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THE KINDS OF BACTERIA FOUND IN RIVER WATER.

By EDWIN OAKES JORDAN, Ph.D.,

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IN the course of a bacteriological examination of river waters during the autumn of 1901, an opportunity occurred for studying an unusually large series of microorganisms. The material derived from comparing a considerable number of cultures—543—is so suggestive that, although the work is not yet complete, it may be of advantage to other workers if the general character of the results be indicated at this time.

The cultures were isolated from three different rivers, the Illinois, the Mississippi, and the Missouri. The nature of the collecting stations may be briefly described.

1. *The Illinois River at Averyville.* As is well known the Illinois River receives a large part of the sewage of Chicago, but by the time the flow reaches Averyville (130 miles below Lockport, the point where the Chicago drainage canal discharges into the Desplaines River[1]), oxidation of the organic matter is practically complete and the bacterial content is not high[2]. The mean monthly discharge of the river at this point was about 7000 cubic feet per second during Oct.—Dec., 1900[3].

2. *The Illinois River at Pekin.* Between Averyville and Pekin, a distance of about six miles, most of the sewage of Peoria (population 56,100, according to the U.S. Census 1900) enters the river, together with a great amount of stockyards waste, distillery slop, and refuse

[1] *Journal Experimental Med.*, 1900, v., p. 271; *Journal of Hygiene*, 1901, i., p. 295; see Map on p. 296.

[2] The average number of colonies per one c.c. during the investigation was 5800, the mean of 67 daily determinations.

[3] Illinois State Board of Health, *Report of the Sanitary Investigation of the Illinois River and its Tributaries*, 1901, p. 179.

from various manufactories. Pollution at this point is therefore both considerable in amount and recent in origin.

3. *The Illinois River at Grafton.* Between Pekin and the mouth at Grafton (143 miles), although several small towns and villages drain into the river, comparatively little additional sewage enters. The oxidation of the Peoria sewage is practically complete when the flow reaches Grafton.

4. *The Mississippi River at Grafton.* There is no considerable pollution for some distance above this point. The volume of water is much greater than that of the Illinois.

5. *The Missouri River at Fort Bellefontaine.* The collection of water was made about twenty-five miles above the mouth of the river and about seven miles above the town of St Charles (population U.S. Census, 1900, 7982).

6—9. *The Mississippi River at the St Louis Water-Works.* At this point, about three miles below the mouth of the Missouri, four samples were taken, nearly in line across the river : one near the Missouri bank, one at the inlet tower of the St Louis Water-Works, one in the channel, and one near the Illinois bank. The water of the Illinois River clings in part to the east (Illinois) shore[1] and that of the Missouri to the west (Missouri) shore, the intermediate body of water being an admixture in varying proportions of water from these two rivers and the Mississippi.

The exact point of collection in all cases corresponded to that chosen for the earlier observations (*op. cit.*). Three laboratories were installed for the work in 1901, one at Peoria, one at Grafton, and one at St Louis. It was found possible to plate the water within about an hour after its collection, except in the case of Pekin (2) and Fort Bellefontaine (5), where an interval of from two to three hours between collection and plating proved unavoidable.

The methods employed for the study of the bacterial cultures were directed toward securing uniform conditions. In the first place, immediately after isolation all the cultures were "rejuvenated" by incubating for three days at 20° C. in nutrient broth of 0·5 acid reaction. Gelatin plates were then made from the broth culture; if only a single species developed, agar tube-cultures were prepared and used as the stock-cultures of the organism. Full data regarding the source of material, date of isolation, etc., were preserved by a

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[1] Cf. *Journ. Experimental Med.*, 1900, v., p. 271.

convenient card-catalogue system. In the second place, special efforts were made to insure a uniform composition of the culture media. The initial study of the cultures was pursued in the University laboratories by several different observers, but the culture media were all prepared by one person, and particular attention was devoted to the details of preparation[1], neutralization, etc.

To these two measures much of the uniformity in the results must be attributed. The method of preliminary broth cultivation in particular, as claimed by Fuller and Johnson[2], undoubtedly helps to place on a common biological level organisms that have been variously affected by aquatic life. The procedure is open to the objection that growth in a common medium may tend to cause convergence in organisms really distinct, and to create a uniformity of type that does not exist in nature. This objection must be admitted to have weight. Until, however, it becomes possible for bacteriologists to frame definite taxonomic rules, the practice of placing bacteria in "groups" rather than in "species" will be found expedient, and the method of rejuvenation, whatever else may be said for it, certainly facilitates such grouping.

The organisms studied in this work have been isolated from four different kinds of culture media: (1) from 48-hour-old gelatin plates incubated at 20° C.; (2) from 48-hour-old dextrose broth fermentation tubes incubated at 37°C.; (3) from litmus-lactose agar plates (35° C.) after passage through carbol broth at 35°C.; (4) from neutral red-broth at 37° C.[3]

It is apparent that in each case a selected flora is obtained. The limiting to two days the period of incubation of the gelatin plates leads, for example, to the isolation only of the more rapidly growing species, and the fermentation tube—when used in the way we have employed it—appears to yield *B. coli* more frequently than other gas-producing species (see Table I). For these reasons, the microorganisms

[1] The general methods of preparation employed in the work were those recommended by the Bacteriological Committee of the American Public Health Association (*Reports and Papers of the Amer. Public Health Association*, Vol. xxiii., p. 60, 1898); with slight modifications:

(*a*) The reaction of the sugar-broths used for the fermentation tests was neutral to phenolphthalein instead of 1·5 acid.

(*b*) The ordinary nutrient gelatin, agar and broth were 1·0 acid.

(*c*) The broth used for both the fermentation and the indol tests was always freed from sugar by Smith's method.

[2] *Journ. Experimental Med.*, 1899, iv.

[3] E. E. Irons, *Journal of Hygiene*, 1902, Vol. ii., p. 314.

enumerated in this paper are not to be considered as representing the whole microbic flora of the river water, but must be looked upon as selected groups which have come to development under the conditions above specified. It must indeed be recognized that no single culture medium will permit the development of all the forms of bacteria actually present in water. The following data, therefore, relate only . to those kinds of bacteria revealed by the particular methods that we have employed.

The characteristics of the different microbes were recorded in the first instance upon record sheets similar to those used by Fuller and Johnson (*op. cit.*), and in most cases the findings were afterwards verified independently by another observer. A definite time limit was set[1]. In this way 543 cultures were studied. The arrangement and classification of this large number of cultures presented peculiar difficulties, and I am especially indebted to Miss Mary Hefferan for assistance in this task. Provisional groupings were first made upon the basis of the characters tabulated on the record sheets (Table III), and a more thorough study was then made of each group, the whole work involving much detail.

The system of group arrangement of water bacteria has been employed by Marshall Ward[2], by Boyce and Hill[3], by Fuller and Johnson[4], and others. It presents many advantages, one of the most obvious being that the more salient biological characters of an organism receive in this way the greatest emphasis. This may not in all cases lead to the most "natural" classification, but it has at least the merit of avoiding much floundering among bewildering synonyms and incomplete descriptions. A more detailed study of each group would doubtless reveal a necessity for further subdivision; in the actual state of classification, however, the writer believes that more is to be gained by compression and unification than by a dispersive arrangement. The different groups considered in the present study may first be enumerated, and then treated separately in more detail.

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[1] Cultures not yielding positive results, *e.g.*, in curdling milk, *within ten days*, are recorded in the tables as negative, *i.e.* by the minus sign.

[2] *Proc. Roy. Soc.*, 1897, LXI. p. 415.

[3] *Journal of Pathol. and Bacteriol.*, 1899, VI. p. 32.

[4] *Journ. Experimental Med.*, 1899, IV. p. 609.

TYPE.

Group   I.   *B. coli communis.*
Group   II.   *B. lactis aerogenes.*
Group   III.   *B. proteus.*
Group   IV.   *B. enteritidis.*
Group   V.   *B. fluorescens liquefaciens.*
Group   VI.   *B. fluorescens non-liquefaciens.*
Group   VII.   *B. subtilis.*
Group VIII.   Non-gas-forming, non-fluorescent, non-spore-forming bacilli which liquefy gelatin and acidify milk.
Group   IX.   Similar to Group VIII., save that the milk is rendered alkaline.
Group   X.   Similar to Group VIII., save that gelatin is not liquefied.
Group   XI.   Similar to Group IX., save that gelatin is not liquefied.
Group   XII.   Similar to Group XI., save that the reaction of milk is not altered.
Group XIII.   Chromogenic bacilli not included in the above groups.
Group   XIV.   Chromogenic *Staphylococci.*
Group   XV.   Non-chromogenic *Staphylococci.*
Group   XVI.   *Sarcinae.*
Group XVII.   *Streptococci.*

Bacilli   Micrococci

                    Staphylococci   Sarcinae   Streptococci
                                           (Group XVI.)   (Group XVII.)

                Chromogenic   Non-chromogenic
                (Group XIV.)   (Group XV.)

Gas producing   Non-gas-producing   Chromogenic
(Groups I.–IV.)   (Group XIII.)

                Fluorescent   Non-fluorescent

Liquefy gelatin   Non-liquef. gelatin
(Group V.)   (Group VI.)

                Liquef. gelatin   Non-liquef. gelatin

        Spore-forming   Non-spore-forming
        (Group VII.)

        Milk acid   Milk alk.   Milk acid   Milk alk.   Milk amphoteric
        (Group VIII.) (Group IX.)   (Group X.)   (Group XI.)   (Group XII.)
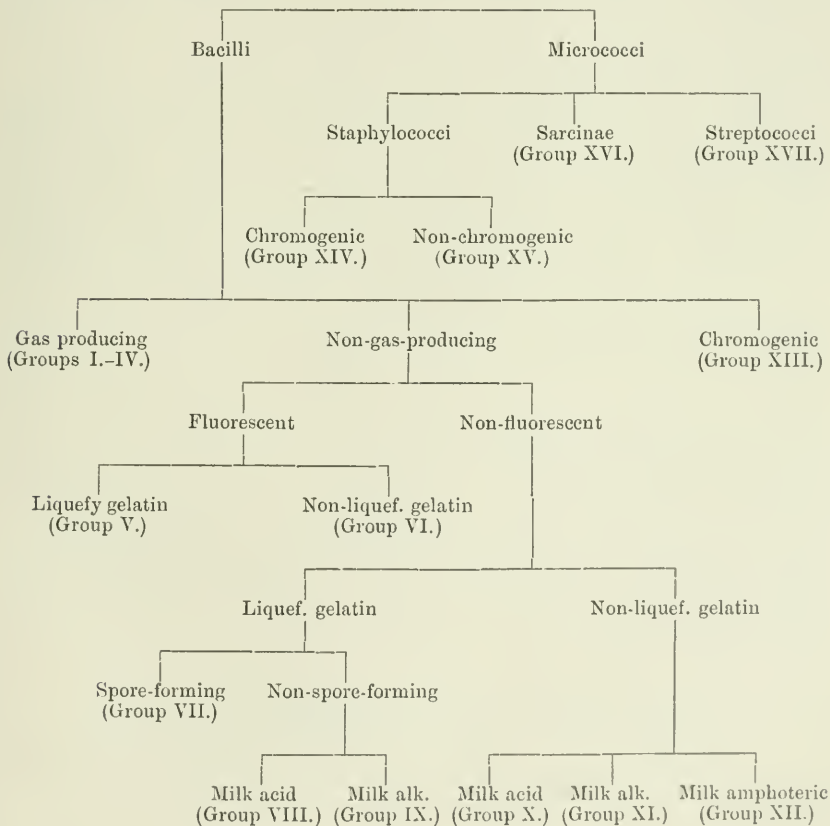
It is not supposed that these groups are of equivalent value or that the characters upon which they are based should have equivalent weight in a scheme of natural classification. It will be noticed both that the tabular key separates quite widely groups of organisms which are in reality closely allied (as Groups I. and X.), and also that certain groups are distinguished from one another on comparatively trivial grounds (as I. and II.), while others are more fundamentally dissimilar. The assignment of proper limits to a group of organisms is, however, a matter for future settlement; convenience of treatment rather than precise and balanced natural affinities must for some time to come be the guiding principle of such grouping. The gas-producing organisms, for example, owing to the prominence that has been accorded them in the sanitary investigations of water, are here given what might be regarded on purely taxonomic grounds a disproportionately detailed consideration. On the other hand large groups of microorganisms are here left in a relatively undifferentiated condition. All perhaps that need be said is that the grouping here used has proved a useful one in dealing with the microorganisms encountered in our work, and is presented simply as a framework upon which to arrange our results.

Group I. The organisms of which this group is composed possess the following characteristics : they are motile bacilli; curdling milk rapidly, with little or no subsequent solution of casein; fermenting dextrose and lactose, and in some instances sucrose also; always yielding gas in dextrose broth with excess of H (approximately $H : CO_2 :: 2 : 1$); showing no liquefaction of gelatin in ten days; usually, but not always, producing indol in marked amount. Type : *B. coli.* Two varieties : (A) fermenting dextrose, lactose and sucrose (variety α, Theobald Smith[1]; *B. coli communior*, Durham[2]), and (B) fermenting dextrose and lactose only (variety β, Smith[3]; *B. coli communis verus*, Durham[4]) were distinguished. Twenty-five cultures of variety (A) were encountered and twenty-one of variety (B). Durham[5] is inclined to think that variety (A) is a commoner inhabitant of the faeces than (B). Moore and Wright[6] in their observations upon *B. coli communis* from certain species of domesticated animals, studied in all forty-three cultures, of which twenty-one fall under variety (A) and twenty-two under variety (B).

[1] *American Journal of Medical Sciences*, Sept., 1895.
[2] *Journ. of Experimental Med.*, 1901, v. p. 368.
[3] *loc. cit.*   [4] *loc. cit.*   [5] *op. cit.* p. 372.
[6] *American Medicine*, 1902, iii. p. 504.

Group II. One of the chief characters distinguishing this group from the preceding is the lack of motility. There are, however, other fairly constant correlated peculiarities. One of these is the more tardy action upon milk, the members of Group I. producing a firm clot within 48 hours, while those placed in Group II. do not as a rule curdle the milk until after three or four days, and in some cultures curdling does not appear until still later. All, however, curdle milk within 10 days. The appearance of the colonies upon a gelatin plate is another feature of differential value. The colonies are less spreading, more convex, fleshy, and with smooth well-defined margins. Type: *B. lactis aerogenes.* Possibly *B. coli immobilis* of some authors belongs here, but with few exceptions all the non-motile bacilli of this general class that have been examined possess together with their lack of motility the correlated characteristics above noted. As in Group I., a variety (*A*) (16 cultures) fermenting dextrose, lactose and sucrose and a variety (*B*) (11 cultures) fermenting dextrose and lactose only, have been found. Variety (*A*) gave a positive indol reaction in 10, a negative in 6 cultures; variety (*B*) a positive in 10, a negative in one.

Groups I. and II. (undifferentiated). A number of cultures were positively identified as belonging to Groups I. and II., but were not differentiated further. Of these, 17 belonged to variety (*A*) and 12 to variety (*B*); 13 cultures of variety (*A*) gave a positive indol reaction and one a negative (3 undetermined); 8 of variety (*B*) a positive and one a negative (3 undetermined).

In tabular form the relations of these groups appear as follows:

	No. of cultures		Indol +	Indol −
Group I.				
Var. (*A*)	25	...	16	9
Var. (*B*)	21	...	20	1
Group II.				
Var. (*A*)	16	...	10	6
Var. (*B*)	11	...	10	1
Groups I. and II. (undifferentiated)				
Var. (*A*)	17	...	13	1
Var. (*B*)	12	...	8	1

Cultures belonging to Var. (*A*), Groups I. and II., 57 %.
Cultures belonging to Var. (*B*), Groups I. and II., 43 %.
Group I., positive indol, 78 %.
Group II., positive indol, 74 %.
Var. (*A*), positive indol, 71 %.
Var. (*B*), positive indol, 92 %.

The fact that a large number of the organisms here placed in Group II. have yielded a positive result with the indol test may be taken to indicate either that grouping on the basis of non-motility is of questionable value or that inability to produce indol is not so constant a characteristic of the *B. lactis aerogenes* group as sometimes supposed. The cultivation of organisms related to this group under conditions favouring proteolytic activity has been shown by Miss Peckham[1] to enhance and even develop the indol-forming power. Possibly the rejuvenation method used in our work may have had a similar effect. In any case, if Groups I. and II. be considered as a whole, it is evident that there is no strict correlation between motility and power of indol production.

Group III. The members of this group ferment dextrose and sucrose, rarely lactose. They are for the most part vigorously proteolytic, rapidly liquefying gelatin and blood-serum, and precipitating and then dissolving casein. The organisms placed in this group fall conveniently into three subdivisions.

1. *Proteus vulgaris* type, always fermenting with gas production dextrose and sucrose, never lactose; liquefying gelatin, casein and blood-serum. Indol is almost always produced (20 positive cultures, 3 negative). Milk is almost always curdled with acid reaction, but the curdling is usually less rapid than in Groups I. and II. and the acidity less intense. All cultures are actively motile. The gas produced in dextrose broth always contains less CO_2 than H (residual gas), and the proportion of H is generally higher than in Groups I. and II.; in 2 cultures out of 23 there was no absorption with NaOH. The total amount of gas formed in the fermentation tube is as a rule somewhat less than that formed by the members of Groups I. and II., as has been noted by Smith[2], but there is considerable variation in this respect.

Widely divergent statements are found in the literature concerning the characters of the species designated as *Proteus vulgaris*[3]. Nearly all writers, for instance, state that milk is both curdled and rendered acid by the bacillus entitled to this name, but Ford[4] has used the term Proteus group for an assemblage of organisms that produce terminal alkalinity in milk and refuse to curdle. A number of varieties, with

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[1] *Journ. Experimental Med.*, 1897, ii. p. 549.
[2] The Fermentation Tube, *Wilder Quarter Century Book*, p. 212.
[3] *B. vulgare*, Lehmann and Neumann, *Bacteriology*, translation by Weaver, p. 295.
[4] *Journ. of Med. Research*, 1901, vi. p. 211.

different gas-producing properties, are included by Ford under this head. Fuller and Johnson[1] include two spore-bearing organisms under their Proteus type, although most writers state that *Proteus vulgaris* is not known to form spores. Marshall Ward[2], in the most thorough study of the group that has been made since Hauser, has described with great detail a number of forms both vigorous and attenuated, but leaves unmentioned the peculiarities of gas-production, although Smith[3] apparently regards the behaviour in the fermentation tube as one of the most conspicuous characters of the Proteus group.

In view of these marked discrepancies and discordant opinions, I have ventured here to limit the name *Proteus vulgaris* to those organisms possessing the gas-producing properties above indicated. The fact that when this is done the majority of the organisms so thrown together show a correlation in other important biological characters is the best evidence that this procedure is not so arbitrary as might appear, but that, in this case, arrangement according to gas-production brings into line organisms possessing a close affinity in other respects.

An interesting tendency to produce yellow pigment is manifested by many cultures belonging to this group. Marshall Ward[4] has already drawn attention to the occurrence of " Yellow Proteus " forms, and has pointed out their probable relationship to such " species " as *B. radiatus* and *B. ochraceus* (Zimmermann), and *B. arborescens* (Frankland). More than half of the cultures that I have placed in the Proteus group showed a more or less pronounced development of pigment on potato or agar, and nearly all imparted a distinct yellow or buff tinge to milk.

2. *Proteus varieties.* In addition to the 23 well-marked cultures placed in subdivision (1), a number of other cultures similar to the type, but differing in certain respects, have been separated from the type chiefly for the purpose of recording their frequency of occurrence. The most common departure from the type is in the direction of proteolytic power. It has been long recognised that the three Proteus varieties originally established by Hauser[5] upon the ground of their different behaviour towards gelatin were in reality most intimately related, and Hauser himself subsequently admitted that the liquefying varieties could be transformed into the non-liquefying[6]. In general,

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[1] *Journ. Experimental Med.*, 1899, iv. p. 609.
[2] *Annals of Botany*, 1899, xiii. p. 197.
[3] *loc. cit.*   [4] *op. cit.*
[5] *Ueber Fäulnissbakterien*, Leipzig, 1885.
[6] *Centralbl. f. Bakteriol.*, 1892, xii. p. 629.

observers of water and soil bacteria have encountered the liquefying member of this group more frequently, and have further noted that loss of liquefying power often followed prolonged cultivation under artificial conditions. Cultures with feeble proteolytic power are, however, occasionally met with among water bacteria. Marshall Ward[1] has carefully described a number of these organisms found in the river Thames, and presumably attenuated by aquatic life.

In the present study several of these aberrant or weakened forms were discovered: (1) essentially like the type, save that blood-serum was not liquefied (4 cultures); (2) like the type, save that blood-serum was not liquefied and casein was very slightly or not at all dissolved (2 cultures); (3) like the type save that casein was not dissolved (3 cultures); (4) like the type save that gelatin was liquefied only after 20—40 days (4 cultures); (5) like the type, save that gelatin was liquefied only after 20—40 days and casein and blood-serum were not dissolved at all (1 culture); (6) like the type save that milk was not curdled, although some acid was formed (2 cultures); (7) like the type save that milk was rendered slightly acid but was not curdled, and casein and blood-serum were not dissolved (1 culture).

3. *B. cloacae.* The organisms placed under this head are characterized by an "inverted gas formula," that is to say, an excess of CO_2 over H in the dextrose fermentation tube. In freshly isolated cultures the proportion of H to CO_2 may be as low as 1 to 5 or 6, but under cultivation there is a tendency for the proportion of H to increase until an approximately stable ratio of 1—2 is reached. I have had cultures under observation for upwards of 3 years, during which period the ratio has remained constant at 1—2. Sucrose is fermented by all cultures, and lactose, although often very slowly, by the majority (14 out of 21 cultures). Considerable variation is shown in the action upon gelatin: 2 cultures liquefying rapidly, 14 slowly, 4 only after 30—40 days, and one not at all. All are actively motile. Milk is curdled by all cultures with acid reaction and the casein is dissolved by 13 cultures. Only 8 cultures out of 21 have produced indol. Blood-serum was liquefied by 5 cultures out of 16 tested.

I have placed this group of organisms together with the Proteus forms, since it seems allied to the Proteus type through morphology, habitat, action upon sugars and proteolytic power. Lactose fermentation with gas production is absent in the Proteus group, and is either

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[1] *op. cit.*

absent or feeble in most members of the *B. cloacae* group. On the other hand the forms included under the name of *B. cloacae* are as a class much less powerfully proteolytic than the Proteus type, and the less frequent occurrence of indol-producing cultures is perhaps to be correlated with this property. Both the Proteus and *B. cloacae* groups contain a number of varieties which in the future it will probably be possible and useful to differentiate fully. The individual organisms composing these groups are themselves quite variable, and the whole group seems to possess a less stable biological equilibrium than many other water bacteria.

Group IV. Bacilli closely related to the colon group, but fermenting dextrose only, never lactose or sucrose. Milk is rendered strongly alkaline and the casein is dissolved. All are actively motile. A compact group of 6 cultures. *B. enteritidis* type. Indol is produced in slight amounts by 5 of the cultures.

Groups V. and VI. Fluorescent Type. Bacteria producing fluorescence were frequently found in the water; in all 58 cultures were isolated and studied, of which 33 were able to liquefy gelatin, while 25 cultures did not possess this power. An interesting feature brought out by the study of this large series is the correlation between the behaviour in gelatin and that in milk. The power of liquefying gelatin was invariably associated with that of coagulating milk, accompanied by a more or less intense acid reaction and rapid peptonization of the casein. This certainly does not accord with the statement of Lehmann and Neumann (*op. cit.*) that *B. fluorescens liquefaciens* never coagulates milk. On the other hand, a strong alkaline reaction, without curdling, was produced in litmus milk by those cultures which did not liquefy gelatin. These correlated reactions, and the fact that all of the non-liquefying cultures were without the ability to grow either in the closed arm of the fermentation-tube or without oxygen (Wright's anaerobe method), while the liquefying forms varied in this respect, served to distinguish the two groups sufficiently. No indication was found of a reacquirement of the power of liquefaction upon subculture, such as was observed by Boyce and Hill[1].

In other characteristics the liquefying and non-liquefying forms were much alike, and the different cultures in each group varied so slightly as to suggest the essential identity of many of the fifty or more extant "species" of green fluorescing bacteria, some of which

[1] *Journ. of Pathol. and Bacteriol.*, 1899, Vol. vi. p. 32; *Thompson Yates Laboratories Reports*, 1898—99, p. 37.

appear to have been distinguished and named from characters not highly significant. The following are examples: *Bacillus scissus* Frankland[1] appears to differ from the original *Bacillus non-liquefaciens* Eisenberg[2] only in reduction of nitrates; *Bacillus putridus* Flügge[3] only in the production of a putrid odour; *Bacillus incognitus* Wright[4] has been distinguished from these because it grew at body temperature, while Wright's[5] *Bacillus fluorescens convexus* and *Bacillus fluorescens foliaceus* show no conspicuous differences. Again, three species described by Ravenel[6], *Bacillus fluorescens ovalis*, *Bacillus striatus viridis* and *Bacillus fluorescens undulatus*, seem to differ only in the size of the bacillus in each case. In the same manner, liquefying "species" have been distinguished on the basis of such uncertain reactions as the reduction of nitrates (cf. Schmolck's *Bacillus fluorescens nivalis[7]*), or by viscidity of growth (cf. *Bacillus viscosus* Frankland[8]).

All 25 cultures of *Bacillus fluorescens non-liquefaciens*, and all but three of the liquefying forms grew at 37° C., although a few developed only feebly, the existence of intermediate forms showing that this character is probably not a constant one. Inconstancy was also shown in the power of producing indol and reducing nitrates under ordinary conditions, some cultures reacting positively at two trials and negatively at the third, although grown under the same conditions and for the same length of time. Unrejuvenated cultures often failed to give a positive reaction, or gave it sporadically in one of a series of cultures. Lack of rejuvenation probably accounts for some of the differences in "species" noted above.

Nearly all varieties produced an unpleasant odour in bouillon, varying with the age and luxuriance of growth; some cultures showed viscidity.

Morphologically, all of the 58 cultures were small bacilli, which varied somewhat in length. All were motile; in fact, very few fluorescent organisms of this type have been described as non-motile, viz., Eisenberg's *B. non-liquefaciens*, Kruse's *B. fluorescens immobilis[9]*, Lustig's *B. aquatilis fluorescens[10]*. Considering the difficulties often hedging the determination of motility, it would seem that observers

[1] *Zeitschr. f. Hygiene*, 1899, Vol. VI. p. 399.
[2] *loc. cit.* p. 145.   [3] *loc. cit.* p. 292.
[4] *Memoirs, National Academy of Science*, 1895, Vol. VII. p. 436.
[5] *loc. cit.*, pp. 438, 439.
[6] *Memoirs, National Academy of Science*, 1896, Vol. VIII. pp. 9, 20, 22.
[7] *Centralbl. f. Bacteriologie*, 1888, Vol. IV. p. 544.
[8] *Zeitschr. f. Hygiene*, 1889, Vol. VI. p. 391.
[9] Flügge, *loc. cit.*, p. 294.   [10] *loc. cit.*, p. 64.

meeting with a non-motile fluorescent form should scrutinize it with particular care in view of the evident rarity of such organisms.

All cultures were tested for the .presence of pyocyanin by treatment with chloroform, but in no case was this pigment found.

Group VII. Spore-forming Bacilli: the Subtilis group[1]. Forty-six cultures were studied which were characterized by the formation of spores. Upon examination they fell into several subgroups: (1) the Subtilis type proper, comprising the majority of the cultures isolated (26). These showed a white and usually dull and wrinkled growth on agar slant, and the characteristic feathery or arborescent growth in the depth of an agar stab culture. All liquefied gelatin rapidly, curdled milk with acid reaction, and reduced nitrates. On potato the growth was dry, white, and later sometimes wrinkled. (2) Fourteen varieties seemed to belong to the mesentericus type. Three of these were distinctly *B. mesentericus fuscus*[2], very dry, yellow-brown, and wrinkled on agar, and showing a raised and exceedingly crumpled 24-hour growth on potato at 37°. Three others were dirty-white on agar, while on potato they produced a dry and rose-coloured growth. One of these even showed touches of deep red in an old, much wrinkled growth on the latter medium. These latter were probably varieties of *B. mesentericus ruber*[3]. Some of the eight remaining varieties of this subgroup would probably be classed with *B. mesentericus vulgatus*[4]. Their whole behaviour, however, points to the conclusion that no sharp line of demarcation can be drawn between the varieties of this latter type and those of *B. subtilis*. The growth of these eight forms on agar was intermediate between the thin, brown, wrinkled appearance of the typical *B. mesentericus fuscus* and the luxuriant, white, often moist growth of the typical *B. subtilis*. The indefiniteness in the affinity of these varieties is also increased by the fact that the occurrence of a wrinkled growth on potato cannot be relied upon to separate them from *B. subtilis*, although the character of such growth was distinctive for the three cultures mentioned above as *B. mesentericus*. As for the other reactions of these forms; all liquefied gelatin rapidly; the majority curdled milk, while others only acidified it; all reduced nitrates; and a few produced indol (like the culture of *B. mesentericus*

[1] Cohn, *Beiträge zur Biologie der Pflanzen*, Vol. I., Heft II. p. 175, 1875 ; also Flügge, *loc. cit.*, p. 196.

[2] Flügge, *loc. cit.*, p. 199.

[3] Globig, *Zeitschr. f. Hygiene*, 1888, Vol. III. p. 322.

[4] Flügge, *loc. cit.*, p. 198.

vulgatus isolated by Fuller and Johnson).   Morphologically the bacilli were not unlike *B. subtilis*.   (3) Four cultures were found of a spore-forming organism in which the vegetative form was a short, thick, non-motile bacillus, of a diameter greater than 1μ, tending to large, round, involution forms.   On agar the growth was yellow and moist; on potato, soft, luxuriant and cream-coloured, later becoming somewhat wrinkled.   They further differed from other spore-bearing forms in not reducing nitrates and in curdling milk with alkaline reaction. (4) One variety was isolated which was morphologically unlike the mesentericus-subtilis type, and conformed more closely to the descriptions of *B. megatherium*[1].   It was a large motile bacillus, "bogig gekrümmt" (Migula).   It gave a smooth, moist growth on agar, liquefied gelatin, reduced nitrates, turned litmus milk slightly alkaline, and slowly decolorised it.   (5) One variety of spore-forming bacillus was found which did not liquefy gelatin. .It was a short, motile bacillus, with oval spores; the growth on agar was moist and smooth; milk was made alkaline.

Group VIII.   Bacilli which liquefy gelatin and acidify milk.   Ward notes in his Group XIV. some rapidly liquefying colourless bacilli, of which he studied 5 varieties "conforming to the type of *B. termo* as amended by Macé[2]."   His series includes Eisenberg's *B. liquefaciens*[3], Frankland's *B. liquidus*[4], Zimmermann's *B. punctatus* and *B. devorans*[5]. He says: "The type is one of the commonest in the Thames and a pronounced putrefactive bacterium."   Boyce and Hill entirely omit this group, which, like Ward, we have found one of the commonest in water.   Our grouping, however, differs from that of the latter author in that the members of his Group XIV. are distributed between our Groups VIII. and IX.

Seventy-four varieties were isolated and their characters tabulated. Nearly all of these—62—coagulated milk in addition to acidifying it; these were further separated into subgroups on the basis of motility, 48 varieties showing independent motility, *i.e.*, possessing flagella. Twenty-four of these motile organisms produced indol, including 6 which also reduced nitrates; limited growth on potato characterized about one-half of these, while the others, in which the indol reaction was negative, were all luxuriant on potato.   This series of 48 cultures was evidently allied to such organisms as *B. albus putridus* Maschek[6];

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[1] Migula, *loc. cit.* p. 516.   [2] *loc. cit.* p. 585.   [3] *loc. cit.* p. 112.
[4] *Zeitschr. f. Hygiene*, 1899, Vol. VI. p. 382.   [5] *loc. cit.* pp. 38, 48.
[6] Chester, *loc. cit.* p. 237.

B. circulans, B. hyalinus, and *B. delicatulus* Jordan [1]; probably also *B. pestifer* and *B. diffusus* Frankland [2]; and *B. sulcatus* Kruse [3].

The 14 non-motile varieties showed the same slight differences as those recorded above; six produced indol, three reduced nitrates, and several did not grow luxuriantly on potato. *Bact. flexuosum* Wright [4] and *B. radiatus* Zimmermann [5] are non-motile organisms of this description. The tan-coloured or yellowish growth which some of the varieties of this group show upon potato seems to connect them to the yellow forms of Group IX.

Aside from the varieties already noted, 12 were recorded which failed to precipitate the casein in milk, although they produced acidity of the medium. The majority of these were motile, and several produced indol or reduced nitrates. Their description coincides broadly with those of *B. superficialis* Jordan [6], *B. antennaeformis* Ravenel [7], *B. radiatum* Chester [8], and *B. inunctus* Pohl [9].

All of the 74 varieties of this group grew well at body temperature; the majority liquefied casein and blood-serum as well as gelatin, and were facultative anaerobes. They are probably closely allied putrefactive organisms. At some points they approximate the Proteus group.

Group IX. Bacilli which liquefy gelatin and produce alkalinity of milk.

Thirty cultures were included in this series. Five of these coagulated milk with alkaline reaction, the soft curd being later peptonized; of these five, two were motile and three non-motile, little difference existing otherwise. Among the remaining 25, were found eighteen motile organisms of the type exemplified by *B. formosus* Ravenel [10], *B. liquidus* Frankland [11], *B. punctatus* Zimmermann [12], *B. liquefaciens* Eisenberg [13], and *B. stoloniferus* Pohl [14]. None of these 18 varieties produced indol, and only 5 reduced nitrates, while nearly all grew at 37° C., liquefied casein and blood-serum, and were luxuriant on potato, upon which the growth was often yellowish or tan-colour. Two of them did not develop on potato, thus corresponding to Zimmermann's description of *B. devorans* [15].

[1] *Report of Mass. Board of Health*, 1890, pp. 831, 835, 837.
[2] *Zeitschr. f. Hygiene*, 1899, Vol. VI. pp. 381, 396. *Phil. Trans. Royal Soc.*, London, 1888, p. 277.
[3] Flügge, *loc. cit.* p. 318.
[4] *loc. cit.* p. 160.   [5] *loc. cit.* p. 58.   [6] *loc. cit.* p. 833.
[7] *loc. cit.* p. 25.   [8] *loc. cit.* p. 162.
[9] *Centralbl. f. Bakteriol.*, 1892, Vol. XI. p. 143; Migula, *loc. cit.* p. 247.
[10] *loc. cit.* p. 12.   [11, 12, 13] *loc. cit.*   [14] *loc. cit.* p. 142.   [15] *loc. cit.* p. 58.

Of the 7 non-motile organisms, two produced indol; in other characteristics they were all like those described above. *B. convolutum* Wright[1] and *B. ambiguum* Chester[2] are non-motile organisms of this type.

Group X.   Bacilli which do not liquefy gelatin but acidify milk.

This and the two following groups comprise together some 91 cultures and correspond to the sixteen forms studied by Ward, and to the twenty studied by Boyce and Hill, and included by these investigators under their Group V. or Coli type. Ward remarks that these forms were common in the river, especially in summer, and states that they showed variation in such characters as production of gas, coagulation of milk, and pathogenicity. Only about half of the cultures examined by Boyce and Hill were capable of producing gas-bubbles in glucose gelatine, and only one formed indol.

That these forms were also found by us to be very abundant is shown by the number isolated. They are, however, separated from the Coli group in our classification by their entire lack of gas production in sugar bouillon; otherwise they showed similar characteristics to those described by Ward.

Of the 32 cultures which are placed in Group VI., only 13 were typically coli-like in their behaviour towards milk. Seven of these, moreover, were non-motile, and of them all only two produced indol, and two produced nitrates. If these, then, are varieties of *B. coli* there is an apparent rough correlation in the absence of four characteristics; the power of causing the free evolution of gas, of reduction of nitrates, of formation of indol, and finally the lack of motility. The absence of indol formation and of motility points perhaps to affinity with *B. lactis aerogenes*. Another series, of which 19 cultures were studied, produced only acidity and not coagulation of milk. Much the greater number (13) of these were non-motile; but on the other hand, a larger number (6) than of the series above, formed indol. (A few of the 19 were not tested for indol.) There is probably no material difference between this series and the foregoing, except that in the latter the amount of acid produced in milk was not sufficient to precipitate the casein.

The motile and non-motile cultures of this type probably contain many forms which have been isolated and named as distinct species. The limited descriptions of most of these forms, especially of the older

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[1] *loc. cit.* p. 460.   [2] *loc. cit.* p. 105.

and the unfamiliar ones, make it impossible to bring them under the classification used here. There are mentioned, therefore, out of a large number of possible forms, only the few for which the description was detailed enough to include statements concerning the gas production and the milk reaction. Of the type that coagulates milk, nearly all the forms falling under this head appear to have been isolated from milk. The majority of these milk varieties appear to be non-motile, e.g. B. punctatus Adametz[1], B. No. 52 Conn[2], and several varieties of B. acidi lactici isolated by Conn[3], which according to him produce no gas and were common in milk; B. limbatum Marpmann[4], B. lacticum Kruse[5], are also from milk, while B. ubiquitus Jordan[6] was isolated from sewage. The motile forms described as coagulating milk are B. No. 107 and No. 137 Conn[7], B. equi intestinalis Dyar and Keith[8], and B. arborescens Ravenel[9]; the forms described as acidifying milk without coagulation are all non-motile, e.g., Conn's B. No. 41, B. No. 54, and B. No. 56[10].

Group XI. Bacilli which do not liquefy gelatin, and which cause alkalinity of milk.

Twenty-nine cultures imparting a marked alkaline reaction to litmus milk were studied. Thirteen of these were motile. None of them formed indol, several reduced nitrates, and nearly all grew well on potato and at 37°. Morphologically the majority of them were alike, small or medium-sized bacilli; one non-motile organism, however, formed long slender filaments, and another was a large bacillus containing dark granules in the protoplasm of each cell.

Among the motile forms described by other investigators which are of this type are B. No. 98 and B. No. 95 Conn[11], B. pinatus Ravenel[12], which forms indol, B. alcaligenes Petruschky[13], while the non-motile forms are B. solitarius and B. geminis Ravenel[14] and B. primus Dyar[15].

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[1] *Landwirthsch. Jahrbücher*, 1899; Chester, *loc. cit.* p. 147.
[2] *Report of Storrs Agric. Exp. Sta.*, 1894, p. 81.
[3] *loc. cit.*, 1899, p. 52.
[4] Flügge, *loc. cit.* p. 366.
[5] Flügge, *loc. cit.* p. 356.
[6] *loc. cit.* p. 830.   [7] *loc. cit.* 1899, pp. 50, 58.
[8] *Mass. Inst. of Tech. Quart.*, VI. p. 3.
[9] *loc. cit.* p. 39.   [10] *loc. cit.* 1894, pp. 57, 82, 83.
[11] *loc. cit.*, 1899 p. 56.   [12] *loc. cit.* p. 32.
[13] *Centralbl. f. Bakteriol.*, 1896, Vol. XIX. p. 187.
[14] *loc. cit.* p. 29.
[15] *Report of the New York Acad. of Science*, 1895, Vol. VIII. p. 360.

Group XII. Bacilli which do not liquefy gelatin and which produce very little or no change in litmus milk. Typhosus Group.

This group comprises a large number of organisms, the majority of which were isolated from gelatin plate colonies. Twelve of these were carefully studied, and it was found that although they were typhosus-like in many of their reactions, they exhibited some slight differences from that type, and differed also among themselves. Eight of them were non-motile, and gave an alkaline end-reaction in dextrose broth, instead of the acid of *B. typhosus.* The majority of them also showed more development on potato than is the case with the typical *B. typhosus.* One variety which was motile, and was morphologically exactly like *B. typhosus*, developed only slowly on agar at 37°, and very feebly if at all in bouillon at that temperature; the agglutination test was negative. Another, which in all its biological reactions was extremely like a typhoid culture, was motile, but was smaller than the typical *B. typhosus.* Unfortunately, this culture was lost before the agglutination test could be applied.

Eighteen other cultures isolated by the gelatin plate method were found unable to produce gas, to liquefy gelatin, or to produce any material change in litmus milk. When these were examined they proved to be without exception non-motile. Fourteen of them were exceedingly fine, slender, transparent bacilli, which did not stain easily, and grew feebly; four were short, thick, almost coccus-like organisms.

Several so-called species isolated from water by different investigators are unquestionably of this type, viz., the *Typhusähnliche* bacilli of Maschek[1] and of Lustig[2], *B. paradoxus* Kruse[3], which, however, forms indol; the several forms of *B. aquatilis sulcatus* I.—V. Weichselbaum[4], which vary morphologically and in growth on potato and at 37°; the non-motile *B. refractans* Wright[5], *B. rodonatum* Ravenel[6], and *B. No.* 55 Conn[7], isolated from milk.

Group XIII. Chromogenic bacilli not included in the above groups.

Bacilli characterized by a marked production of pigment, and not classed on other grounds with the Proteus or other groups were separated into the following subgroups: *A*, Red Chromogenic Bacilli, *B*, Orange Chromogenic Bacilli, *C*, Yellow Chromogenic Bacilli. As the norms for

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[1] *Untersuch. des Leitmeritzer Trinkwassers*, Leitmeritz, 1887.   Migula, *loc. cit.* p. 730.
[2] *loc. cit.* p. 18.   [3] Flügge, *loc. cit.* p. 373.
[4] *Das Oesterreichische Sanitätswesen*, 1889, Nos. 14—23.   Migula, *loc. cit.* p. 731.
[5] *loc. cit.* p. 442.
[6] *loc. cit.* p. 40.   [7] *loc. cit.* 1894, p. 83.

these three colours there have been taken for red, *B. prodigiosus*, for orange, *Sarcina aurantiaca*, for yellow, *Sarcina lutea*.

A. *Red Chromogenic Bacilli*. Two varieties were studied. One of these strongly resembled *B. prodigiosus*, except that its pigment at room temperature was more violet, like that produced by *B. ruber balticus*[1] at 37°. It also differed from *B. prodigiosus* and from *B. ruber balticus*, *B. ruber indicus*[2], *B. plymouthensis*[3], and *B. miniaceus*[4] in producing no gas in dextrose bouillon. It grew rapidly, with luxuriant pigment, which extended throughout the closed arm of the fermentation tube, although no pigment was produced in the entire absence of oxygen.

The other variety was studied from two separate isolations, the cultures proving identical except for slight differences in rapidity of growth and colour of pigment. It differs from the red forms above named in the production of alkalinity in milk, in its non-motility and its very slow liquefaction of gelatin. It did not develop in the closed arm of the fermentation tube, and produced no gas. The pigment was bright rose-red.

An extremely short bacillus that occurred twice was in cultural features almost indistinguishable from the pink chromogenic coccus described under Group X.

B. *Orange Chromogenic Bacilli*. Of the 7 varieties examined, all liquefied gelatin, and grew at body temperature. Two of these varieties were thin, non-motile rods, which in old cultures often showed long bent spirilla forms. They produced a dark-orange pigment, liquefied gelatin only slowly, and turned litmus milk alkaline. These are probably of the type *B. fulvus* Zimmermann[5]. Another non-motile organism curdled milk without acidity, produced indol, and liquefied gelatin rapidly. The other four varieties were motile, and acidified milk with coagulation; two of them produced indol. These forms might possibly be classed with such forms as *B. arborescens* Frankland[6], and *B. aurescens* Ravenel[7].

C. *Yellow Chromogenic Bacilli*. These cannot be definitely distinguished from the last-named subgroup, owing to the variability of both orange and yellow pigments and the occurrence of many

[1] Laurent, *Ann. de l'Institut Pasteur*, 1890, Vol. iv. p. 465.

[2] Flügge, *loc. cit.* p. 302.

[3] Fischer, *Zeitschr. f. Hygiene*, 1887, Vol. ii. p. 74.

[4] Zimmermann, *loc. cit.* p. 46.

[5] *Ibid.* p. 44.

[6] *loc. cit.* p. 482.   [7] *loc. cit.* p. 8.

nondescript intermediate forms.   However, 7 varieties were studied and recorded as yellow.   Three of these liquefied gelatin, one proving to be a typical *B. lactis erythrogenes* Hueppe[1], with yellow pigment and red fluorescence.   The other two were slow liquefiers, and rendered milk slightly alkaline, but differed from each other in motility and in the power of growth on potato.   Of the yellow liquefying bacilli already described, *B. lutescens* Lustig[2], *B. aquatilis* Frankland[3], and *B. helvolus* Zimmermann[4] are non-motile, and have a limited growth or none at all on potato.

Four cultures which did not liquefy gelatin were studied.   Two of these curdled milk with rapid and complete liquefaction of the precipitated casein; they were motile, medium-sized bacilli, possibly related to *B. radiatus* and *B. ochraceus* Zimmermann[5].   Fuller and Johnson seem to be alone in describing the latter organism as large and spore-forming; such characters would relate it to the varieties which have here been classed as of the yellow Subtilis type.   Other writers describe *B. ochraceus* as less than 1μ in diameter and sporeless.   Ward, and Boyce and Hill connect certain yellow non-liquefying forms, which they identify as *B. radiatus* and *B. ochraceus* Zimmermann and *B. arborescens* Frankland, with the Proteus group.   The cultures here treated have been separated from that group chiefly because of their non-production of gas.   In many respects they are closely related to it.

Group XIV.   Chromogenic Micrococci.

Of these, 9 yellow and 5 red forms were worked out in detail. The most striking of the yellow varieties was one which produced luxuriant bright pigment like that of *Sarcina lutea*, which it resembled in cultural features.   It was, however, a larger coccus and showed no packet grouping.   It is probably identical with the variety isolated once from the Thames by Ward, and described in his Group XX. Like three others of the six liquefying varieties observed it coagulated and acidified milk.   Of the other two varieties one grew in milk with production of alkali, the other with no effect upon the medium; the latter may have been a weaker variety of the one first mentioned, to which it was otherwise similar except that it showed no growth on potato.

Three non-liquefying cultures were alike in producing alkali in milk.   All of the yellow forms grew at body temperature.

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[1] Grotenfelt, *Fortschritte d. Medizin*, 1889, Vol. VII. p. 41.
[2] *loc. cit.* p. 781.   [3] *Zeitschr. f. Hygiene*, Vol. VI. p. 381.
[4] *loc. cit.* p. 52.   [5] *loc. cit.* pp. 58, 60.

It seemed impossible to relate these to the yellow cocci isolated by other investigators because of incomplete descriptions. Migula (*System der Bakterien*) gives 14 liquefying and 8 non-liquefying forms.

A coccus which produced a pink pigment appeared very frequently in the water of the Illinois River at Grafton, at times forming from 50 to 90 per cent. of the colonies on gelatin plates. This organism was isolated and exhaustively studied on five separate occasions, showing always the same characteristics. It grew slowly without liquefaction on gelatin plate, luxuriantly on agar, was alkaline in milk, and failed to develop on potato or anaerobically. For a complete description of this organism, as first isolated in 1899 from the Mississippi River, see a paper by Miss Mary Hefferan[1].

Group XV. Non-chromogenic Micrococci.

A. The great majority—27 out of 35—of the colourless coccus forms isolated from time to time were non-liquefying varieties. Of these, 7 different cultures proved to be alike, *i.e.* alkaline in litmus milk with peptonization, growth at 37°, and luxuriant growth on potato.

Only a few cocci have been described as rendering litmus milk alkaline, viz., Conn's *No.* 85[2] and *No.* 47[3] (*M. nivalis* Chester). Cohn's *M. candidus*[4], *M. aquatilis* Vaughan[5], and *M. aquatilis* Bolton[6] may be of the same type.

Thirteen cultures were found to render litmus milk slightly acid in 10 or 15 days, but not enough so for coagulation. With 5 others the milk was amphoteric during 15 days, although microscopic examination showed that development took place. All but 3 of these 16 grew at body temperature, 2 of them reduced nitrates, and they usually grew luxuriantly on potato. The whole series, so far as the tabular reactions show, belonged to the type *M. candicans* Flügge[7], like those mentioned by Ward in his Group XIX. They have not been under observation long enough to determine whether any liquefying power was acquired after long-continued cultivation, as Ward observed with his series.

B. The 8 liquefying forms showed slight differences, which may have been due to variation in vigour. All of them acidified and 4

[1] *Botan. Gazette*, 1900, Vol. xxx. p. 261.
[2] *Report of Storrs Agric. Exp. Sta.* 1894, p. 28.
[3] *loc. cit.* p. 80.   [4] *loc. cit.*, Vol. i. p. 160, 1875.
[5] *Am. Jour. Med. Science*, 1892.
[6] *Zeitschr. f. Hygiene*, 1886, Vol. i. p. 94.   [7] *loc. cit.* p. 117.

coagulated milk; 5 of them also peptonized casein and blood-serum. The others seemed less luxuriant varieties, those which acidified milk without coagulation also refusing to develop at body temperature. Type: *M. coronatus* Flügge[1], *M. acidi lactis* Kruger[2], *M. simplex* Wright[3].

Group XVI.   Sarcinae.

Three cultures were isolated, one being in most respects a typical *Sarcina lutea*, forming a bright yellow pigment, and producing slow liquefaction in gelatin, but with no effect upon milk.   The other two were white forms which did not peptonize gelatin, and which made litmus milk alkaline.

Group XVII.   Streptococci.

In examining the forms described in Group XI., short chains of 3 or 4 cocci were often seen, and 4 varieties showed such well-marked and constant chains in fresh agar or bouillon cultures that they have been definitely separated as streptococci.   The possible significance of the occurrence of *Staphylococci* and *Streptococci* in water was first pointed out by Houston in the report of the Medical Officer to the Local Government Board of London, 1898—1900[4].   From experiments extending over several years Houston concludes that micrococci are readily demonstrable in polluted water and sewage, and that the presence of *Streptococci*, a class of germs unlikely to persist long outside the animal body, seems always to coincide with "animal pollution of extremely recent and therefore specially dangerous kind."   Several investigators, among them Horrocks[5] in England, and recently Winslow[6] in America, consider with Houston that *Streptococci* are typical sewage forms, although it is probable that some of these may be non-pathogenic varieties that are able to exist for some time outside the animal body, and hence may not always indicate recent contamination.

No special method of search for *Streptococci* was employed in this investigation; it is therefore interesting to note that two varieties, the non-liquefying type of Houston and Horrocks and a second type, found also by Winslow[6] (differing from the other only in liquefaction of gelatin), appeared in the Illinois River at Pekin, where the great majority of *Staphylococcus* forms also occurred.   These *Streptococci*

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[1] *loc. cit.* p. 178.   [2] *Centralbl. f. Bakteriol.*, 1890, Vol. VII. p. 495.

[3] *loc. cit.*, 1895, Vol. V. p. 32.

[4] Houston, *Parliamentary Blue Books*, 1898, 1899, 1900, Supplem. XXVIII.

[5] Horrocks, *Bacteriological Examination of Water*; London, 1901.

[6] Winslow and Hunnewell, *Science*, May 23, 1902.

were isolated from culture plates after use of the carbol broth method; two other cultures, both non-liquefying, were isolated from the Mississippi River, Missouri shore, St Louis, by the fermentation tube. All of these organisms grew at 37°, and developed rather better in the depths than on the surface of agar stab cultures; in agar slant cultures they showed a typical transparent growth of small round colonies. They all curdled milk with acid reaction; the variety which liquefied gelatin also rapidly peptonizing the precipitated casein. None of these forms produced indol or reduced nitrates.

The material in this paper, which is itself a condensed summary, does not readily lend itself to a final summing-up, but a few of the points that it illustrates may be noted.

Summary.

1. The kinds of bacteria that are isolated by the gelatin plate method from certain river waters freshly polluted with sewage are different from those found in the same water collected a long distance below the point of pollution.

2. In the freshly polluted river water non-chromogenic *Staphylococci* were found much more abundantly than in the purer waters.

3. In the freshly polluted water the fluorescent bacteria and a group of non-gas-producing, non-liquefying bacteria (Group XI.) were less abundant than in the purer waters.

4. A larger proportion of organisms belonging to the *Proteus* group were isolated from gelatin plates than from fermentation tubes. The reverse is true of the *B. coli* and *B. lactis aerogenes* types. A certain selective influence even upon gas-producing organisms would seem from this to be exerted by the conditions within the fermentation tube.

5. The study of a rather large number of separately isolated cultures belonging to the class of fluorescent microorganisms shows that the differences between the 'liquefying' and 'non-liquefying' varieties are more constant than is sometimes assumed. The action of these forms upon milk is just as diagnostic as their action upon gelatin. All the strains of fluorescent bacteria that were encountered (58) proved to be motile.

6. Considering as a whole the various physiological tests applied to the several groups of microorganisms, it is found that within almost

every group as constituted in the accompanying tables divergence is shown by closely allied organisms in respect to indol formation and reduction of nitrates. The formation of a surface pellicle on broth is also a phenomenon that presents no apparent correlation with more salient physiological characteristics.

TABLE I.

Group	Gelatine Plate	Fermenta- tion Tube	Carbol Broth	Neutral Red Broth	Total
B. coli communis	7	23	11	5	46
B. lactis aerogenes	1	11	11	4	27
B. coli communis and lactis aerogenes	2	7	8	12	29
Proteus	23	9	3	5	40
B. cloacae	3	10	2	6	21
B. enteritidis		4	1	1	6
B. fluor. liq.	26	4	2	1	33
B. fluor. non-liq.	15	5		5	25
B. subtilis	18	4	4	20	46
B. gelat. liquef.					
milk acid	50	7	4	13	74
milk alk.	22	4	2	2	30
B. non-gelat.-liquef.					
milk acid	15	2	3	12	32
milk alk.	26	1		2	29
milk amphot.	29			1	30
Chromogenic Bac. [1]	17				19
Chrom. Staphylococci	11	1	2		14
Non-chrom. Staphylococci	28	1	3	3	35
Sarcinae	2		1		3
Streptococci			2	2	4
	295	93	59	94	543

[1] Two cultures of the red chromogenic group are without data as to place or mode of isolation.

TABLE II.

Type	Illinois River			Mississippi River					Missouri River	Total
					Chain of Rocks					
	Averyville	Pekin	Grafton	Grafton	Missouri Shore	St Louis Intake	Channel	Illinois Shore	Fort Belle-fontaine	
B. coli communis	3	22	1	7	4	2	1	3	3	46
B. lactis aerogenes	7	8	1	5		3	1	1	1	27
B. coli communis and B. lactis aerogenes			1	7	4	1	2	3	2	29
Proteus	6	12	1	1	2	3	1	2	2	40
B. cloacae	13	4	5		2	4		2		21
B. enteritidis	5	1	5		1					6
B. fluor. liq.	3	2	7	9	1	1	2	3		33
B. fluor. non-liq.	8	3	2	2	4	6	5	1	2	25
B. subtilis	7	1	12	4	4		4	2	6	46
B. gelat.-liquef. milk acid	6	11	10	15	4	6		1		74
milk alk.	19	10	7	2	2	1		1	8	30
B. non-gelat.-liquef. milk acid	7	8	2		3	2	6	4		32
milk alk.	6	1	1		2	1	4	2	1	29
milk amphot.	17	6	2	1	2	1		1	2	30
Chromogenic Bac.[1]	17	3	1	1	1	1	1	1		19
Chrom. Staphylococci	7	2	5	1	2	1	1	1		14
Non-chrom. Staphylococci	2	17	3	1	2		4	1		35
Sarcinae	4			2				1	2	3
Streptococci	2	2		1				1		4
	139	115	65	58	40	33	32	29	30	543

[1] Two cultures of the red chromogenic group are without data as to place or mode of isolation.

TABLE III.

GROUPS	No. of cultures	Bacillus	Diameter greater than 1μ	Motile	Spores	Scum	Turbidity	Dull	Wrinkled	Characteristic appearance	Deep funnel	Surface growth	Needle growth	Visible	Luxuriant	Growth in closed arm
Group I. B. coli, var. (a)	25	+	−	+	−	±	+	−	−	−	−	+	+	+	+	+
var. (b)	21	+	−	+	−	±	+	−	−	−	−	+	+	+	+	+
Group II. B. lactis aerogenes, var. (a)	16	+	−	−	−	±	+	−	−	−	−	+	+	+	+	+
var. (b)	11	+	−	−	−	±	+	−	−	−	−	+	+	+	+	+
Groups I. and II. undifferentiated further	29	+	−	±	−	±	+	−	−	−	−	+	+	+	+	+
Group III. (1) Proteus vulgaris (type)	23	+	−	+	−	±	+	−	−	±	+	+	+	+	±	+
(2) Proteus (varieties)	17	+	−	+	−	±	+	−	−	±	±	+	+	+	±	+
(3) B. cloacae	21	+	−	+	−	±	+	−	−	±	±	+	+	+	±	+
Group IV. B. enteritidis	6	+	−	+	−	±	+	−	−	−	−	+	+	+	−	+
Group V. Fluorescens liquefaciens	33	+	−	+	−	+	+	−	−	−	+	+	+	+	+	+
Group VI. Fluorescens non-liquefaciens	25	+	−	+	−	+	+	−	−	−	+	+	+	+	+	−
Group VII. Subtilis	26	+	−	+	+	+	+	−	+	+	+	−	−	+	+	+
Mesentericus vulgatus	8	+	−	+	+	+	+	−	+	+	+	−	−	+	+	+
,, fuscus	3	+	−	+	+	+	+	−	+	+	+	−	−	+	+	−
,, ruber	3	+	−	+	+	+	+	−	+	+	+	−	−	+	+	+
Yellow Subtilis	4	+	+	+	+	+	+	−	−	−	+	−	−	+	+	+
Megatherium	1	+	−	+	+	+	+	−	−	−	+	−	−	+	+	+
Non-liquefying	1	+	−	+	+	+	+	−			−	+	+	+	+	+
Group VIII. Gel. liquef.-Milk acid	74	+	−	±	−	+	+	−	−	−	+	+	+	+	±	+
Group IX. Gel. liquef.-Milk alkaline	20	+	−	±	−	+	+	−	−	−	+	+	+	+	+	+
		+	−	±	−	+	+	−	−	−	+	+	+	±	±	+
Group X. Gel. not liquef.-Milk acid	32	+	−	±	−	+	+	−	−	−	−	+	+	+	+	+
Group XI. Gel. not liquef.-Milk alkaline	29	+	−	±	−	+	+	−	−	−	−	+	+	+	+	+
Group XII. Gel. not liq.-Milk amphoteric	30	+	−	±	−	+	+	−	−	−	−	+	+	+	±	+
Group XIII. Chromogenic Bacilli Red	1	+	−	+	−	+	+	−	−	+	+	+	+	+	+	+
	2	+	−	−	−	+	+	−	−	+	−	+	+	+	+	−
	2	+	−	−	−	+	+	−	−	+	−	+	+	−	−	−
Orange	4	+	−	+	−	+	+	−	−	−	+	+	−	+	+	+
	3	+	−	−	−	+	+	−	−	−	−	+	+	+	−	−
Yellow liquefying	6	+	−	±	−	+	+	−	−	−	+	+	±	±	±	±
Lact. erythrogenes	1	+	−	−	−	+	+	−	−	−	+	+	+	+	+	+
Group XIV. Chromogenic Cocci Yellow liquefying	1	−	−	−	−	−	+	−	−	−	+	+	+	+	+	−
	5	−	−	−	−	−	+	−	−	−	+	+	+	+	+	±
Yellow non-liquefying	3	−	−	−	−	+	+	−	−	−	−	+	+			−
Pink	5	−	−	−	−	+	+	−	−	−	−	+	+	−	−	−
Group XV. Non-chromogenic Cocci Liquefying	8	−	−	−	−	±	+	−	−	−	+	±	±	+	±	±
Non-liquefying	27	−	−	−	−	±	+	−	−	−	−	+	+	+	+	±
Group XVI. Sarcinae Yellow	1	−	−	−	−	+	+	−	−	−	−	+	+	+	−	−
White	2	−	−	−	−	−	+	−	−	−	−	+	+	+	+	+
Group XVII. Streptococci Liquefying	1	−	−	−	−	−	−	−	−	−	−	+	+			
Non-liquefying	3	−	−	−	−	−	+	−	−	−	−	+	+			+

Table III.

BIOLOGY

Biochemical Features

Grows at body temperature	Facultative anaerobe	Affected by range of reaction	Liquefaction			Gas production			Nitrate reduced	Indol produced	Milk			Fecal odour	Nutrient agar tubes	
			Gelatin	Casein	Blood serum	Dextrose broth	Lactose broth	Saccharose broth			Curdled	Acid	Alkaline		Chromogenesis	Fluorescence
+	+	+	−	−	·−	+	+	+	±	±	+	+	−	±	−	−
+	+	+	−		−	+	+	−	±	±	+	+	−	±	−	−
+	+	+	−	−	−	+	+	+	±	±	+	+	−	±	−	−
+	+	+	−	−	−	+	+	−	±	±	+	+	−	±	−	−
+	+	+	−	−	−	+	+	±	±	±	+	+	−	±	−	−
+	+	+	+	+	+	+	−	+	±	±	+	+	−	±	±	−
+	+	+	±	±	±	+	−	+	±	±	±	+	−	±	±	−
+	+	+	±	±	±	+	±	+	±	±	+	+	−	±	−	−
+	+	+	−	+	+	+	−	−	±	±	−	−	+	±	−	−
+	±	+	+	+	+	−	−	−	±	±	+	+	−	±	−	+
±	−	+	−	+	+	−	−	−	±	±	−	−	+	±	−	+
+	−	+	+	+	±	−	−	−	+	−	+	+	−	−	−	−
+	−	+	+	+	+	−	−	−	+	−	±	+	−	−	−	−
+	−	+	+	+	+	−	−	−	+	−	+	+	−	−	brown	−
+	−	+	+	+	+	−	−	−	+	−	+	+	−	−	rose	−
+	−	+	+	+	+	−	−	−	−	−	+	−	+	−	yellow	−
+		+	+	+	−	−	−	−	+	−	−	−	+	−	−	−
+			−	−		−	−	−	−	−	−	−	+	−	−	−
+	+	−	+	+	+	−	−	−	±	±	±	+	−	−	−	−
+	+	−	+	+	+	−	−	−	±	±	+	−	+	−	−	−
±	+	−	+	+	+	−	−	−	±	±	−	−	+	−	−	−
+	+	−	−	−	−	−	−	−	±	±	±	+	−	−	−	−
+	+	−	−	±	−	−	−	−	±	−	−	−	+	−	−	−
±	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
+	+	+	+	+	−	−	−	−	+	−	+	+	−	−	red	−
+	−	+	+ s	−	−	−	−	−	−	−	−	−	+	−	red	·
+	−	−	+	−	−	−	−	−	+	−	−	−	+	−	red	−
+	−	−	+	+	−	−	−	−	−	−	+	+	−	−	orange	−
+	−		+	+	−	−	−	−	−	+	+	+	−	−	orange	−
+	+	+	± s	+	−	−	−	−	±	−	±	−	±	−	yellow ·	−
+	+	·+·	+	+	−	−	−	−	−	−	−	−	+	−	yellow	+ red
+		+	+ r	−	−	−	−	−	−	−	−	−	+	−	bright yel.	−
+	−	+	+	+	−	−	−	−	+	±	+	+	−	−	yellow	−
+			−	+	−	−	−	−	−	−	−	−	+	−	yellow	−
+	−	−	−	+	−	−	−	−	+	−	−	−	+	−	pink	−
+	±	±	+ r	±	−	−	−	−	−	−	+	+	−	−	−	−
+	±	±	−	±	−	−	−	−	−	−	−	−	+	−	−	−
+		−	+ s	+	+	−	−	−	−	−	−	−	−	−	yellow	−
+		−	−	−	−	−	−	−	+	+	−	−	+	−	−	−
+			+	+	−	−	−	−	−	−	+	+	−	−	−	−
+			−	−	−	−	−	−	−	−	±	+	−	−	−	−

STUDY OF THE BACTERIOLYTIC SERUM-COMPLEMENTS IN DISEASE: A CONTRIBUTION TO OUR KNOWLEDGE OF TERMINAL AND OTHER INFECTIONS.

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It has long been known that individuals who suffer from such chronic diseases as nephritis and cirrhosis of the liver are very liable to develop an acute infection during the last stages of their illness. These patients rarely die of their chronic ailment. Often in the very last days, or perhaps hours, of the disease pneumonia, a dysentery, an acute endocarditis, or a streptococcus infection sets in, which quickly terminates fatally and must be regarded as the immediate cause of death. So familiar is this course of events that such pneumonias or dysenteries are now known as a fairly definite group of infections, namely, terminal infections. Often they are not recognized before death, but at autopsy their frequency and varying characters have been well shown by Flexner[1] in his statistical and experimental study upon the subject; and though in some cases of terminal septicaemia the portal of entry was not easily found, yet it is important to note how frequently such local infections as leg ulcer or tonsilitis served as a starting point for the general invasion of bacteria. Of still more importance are his observations upon the bactericidal action of the blood of patients who have chronic diseases. The serum from six out of nine such patients showed a distinct decrease in its destructive power toward the *Staphylococcus aureus*, and two of the three patients whose serum did not show a diminished bactericidal action left the hospital improved.

Since these observations, the work of Ehrlich, Morgenroth, and

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[1] Flexner. *Journal of Experimental Medicine*, 1896, i. p. 21.

Bordet has laid bare the mechanism by which the body protects itself against foreign cells; and it is only necessary to state here that the protective substances of the blood consist in two elements—the intermediary or immune body (amboceptor)—a specific substance, stable at a temperature of 58° C.; and the complement, or alexin—a labile substance, present in all normal sera, but easily destroyed at temperatures above 58° C. For the dissolution of any foreign cells, whether bacteria or red blood corpuscle, both these substances must be present in the serum; therefore, if any individual's blood is bacteriolytic or haemolytic, that person's serum must contain both intermediary body and complement for bacteria or red blood corpuscles.

Ehrlich and Morgenroth[1] have found further that the intermediary body and complement are entirely independent of one another, and may exist in the same serum in different amounts. Under certain conditions the intermediary body remains unchanged, while the complement is decreased. Ehrlich cites an experiment where the haemolytic complement was entirely absent in the serum of a rabbit poisoned by phosphorus, and Metalnikoff[2] has observed the loss of spermolytic complement in the serum of an artificially immunized rabbit which had developed an abscess during the process of immunization. The experiments of Abbott and Bergey[3] have proven conclusively that a marked diminution in the haemolytic complement-content of the blood of rabbits results from the alcoholization of these animals, and the authors have furthermore demonstrated the impossibility of immunizing alcoholized rabbits against foreign red blood corpuscles. The latter phenomenon they believe is due to the decrease in complement, which thereby robs the animal of its power to overcome the toxic effects of foreign cells.

Not only may the complement be decreased, but it may by certain artificial means be increased. This increase in the complement has been effected by injections of various substances, as for instance peptone, salt solution, bouillon, or serum (Wassermann,[4] Müller,[5] Nolf[6]). Moro[7] has found besides that the serum of sucklings is more highly bacteriolytic than the serum of weaned infants.

[1] Ehrlich and Morgenroth. *Berliner klin. Wochenschrift*, 1901, xxxi. p. 683.

[2] Metalnikoff. *Ann. de l'Institut Pasteur*, 1900, xiv. p. 580.

[3] Abbott and Bergey. *Univ. of Pennsylvania Med. Bulletin*, 1902, xv. p. 186.

[4] Wassermann. *Zeitschrift f. Hygiene*, 1901, xxxvii. p. 197.

[5] Müller. *Centralbl. f. Bacteriol. u. Parasitenk.*, 1901, xxix. p. 175.

[6] Nolf. *Ann. de l'Institut Pasteur*, 1900, xiv. p. 297.

[7] Moro. *Jahrb. f. Kinderheilkunde*, 1902, lv. p. 396.

The important part which the complement plays in protecting animals against artificial infections has been beautifully demonstrated by Wassermann[1]. Typhoid bacilli were inoculated into the peritoneal cavity of guinea-pigs. The result was rapid destruction of the typhoid bacilli and complete recovery of the animals. If, however, anti-complement were injected together with the typhoid bacilli, the dose which before had been harmless now became rapidly fatal. Again, in his article on "Infection and Auto-infection," Wassermann[2] suggests that the so-called lowered resistance of certain individuals which renders them so susceptible to infections of various kinds can be explained by a decrease in the complement-content of their blood, and the same hypothesis, he thinks, may account for the development of auto-infections. Supporting this theory are the observations of Neisser and Doering[3], confirmed later by Laquer[4], who found a decrease in the haemolytic action of the blood in uraemia. These authors explain their findings on the assumption that an auto-anti-complement is formed in the blood during uraemia.

Quite recently Hedinger[5] has reported several cases of uraemia in which the blood serum showed a suspension of haemolysis, sometimes only lasting during the period when the symptoms of uraemia were most pronounced.

It is evident, then, that the complement is capable of very great variations from the normal, the intermediary body remaining meanwhile unchanged. The complement represents the more sensitive portion of the serum. It is influenced by a variety of conditions—it may be decreased, it may be increased—but under all these conditions the intermediary body remains a constant quantity. And since the complement is of the most vital importance for the protection of the body against infections, its destruction or even decrease may be attended with the utmost danger to the life of the organism.

At the suggestion of Dr Flexner a series of experiments was made to determine whether in the latter stages of chronic diseases there is a perceptible decrease in the bacteriolytic action of the blood, and, if this is true, to discover what changes take place in the serum to account for the reduction. The serum of seventeen such cases was examined,

[1] *Loc. cit.*

[2] Wassermann. *Deutsche med. Wochenschrift*, 1902, xxviii. p. 117.

[3] Neisser and Doering. *Berliner klin. Wochenschrift*, 1901, No. 22, p. 593.

[4] Laquer. *Deutsche med. Wochenschrift*, 1901, No. 43. p. 74.

[5] Hedinger. *Deutsches Arch. f. klin. Med.*, 1902, lxxiv. p. 24.

and included uraemia, cirrhosis of the liver, chronic pericarditis, and chronic endocarditis.

Methods. The blood was drawn from one of the arm veins, usually by means of a large syringe or by an apparatus devised by Dr Crampton, of the Ayer Laboratory. This apparatus consists of a small glass flask provided with a straight nozzle, 2 cm. in length, which extends out from the side of the flask just below the mouth. Over the nozzle fits a short, stout rubber tube supplied with a needle at the free end. The mouth of the flask was plugged with cotton, and the flask, rubber tube, and needle sterilized. When it was desired to draw the blood, a long rubber tube was fitted over the mouth of the flask and a large aspirating syringe attached to the other end. The needle was then put into a vein, and, by the action of the exhaust syringe, blood was drawn directly from the vein into the sterile flask, where it was allowed to remain for from twelve to eighteen hours until the serum separated from the clot. In every case the skin of the arm was carefully cleansed before the operation. When venesection was thought necessary the blood was caught in a sterile flask as it spouted from the vein.

For the experiments 25 to 150 c.c. of blood was used, the serum being allowed to separate out in the ice-box, and in from eighteen to twenty hours after the blood was taken the clear supernatant fluid was drawn off by means of sterile pipettes. Control cultures were always made from this serum, but in no instance was a growth of bacteria obtained. The bacteriolytic action of the serum was then tested upon two organisms—*B. coli* and *B. typhosus*—the same strain of both organisms being used throughout the experiments. Emulsions from eighteen-hour agar slants were made in normal salt solution, and one loopful (a standard platinum loop) from each salt suspension plated for control. Six loopfuls of each salt suspension were then added to 1 c.c. of unheated complement-containing serum, and three loopfuls of this mixture plated immediately. The mixture was at once put in a thermostat at 36·5° C., and at intervals of one, five, and twenty-four hours, three loopfuls were again plated. The Petri dishes were kept at 36·5° C. for at least twenty-four hours, when the number of colonies on each plate was estimated by means of a piece of plate glass, ruled into small squares, the colonies if numerous being counted under the No. 3 ocular. In spite of certain difficulties, such as the formation of a single colony by agglutinated bacilli, the method proved surprisingly accurate when as controls two or three sets of plates were made from the same tube of serum.

To estimate the reactivating power of a given serum, several cubic centimetres of serum were heated for one hour at 56° to 58° C., and to each cubic centimetre of heated serum containing intermediary body but no complement varying amounts of unheated or fresh serum containing complement were added. The remaining technical procedures were the same as above. By using from 1/20 to 8/10 c.c. of unheated serum containing complement the smallest quantity was determined which sufficed to reactivate 1 c.c. of heated serum (amboceptor)—that is, give sterile plates in twenty-four hours.

The first experiments were made with the serum of normal individuals. Seven different healthy sera were tested; but as might be expected, the results from all the cases were not absolutely in accordance. In every instance, however, 1 c.c. of unheated active serum gave complete bacteriolysis with both organisms in twenty-four hours, and often with typhoid bacilli in five hours. The most noticeable variations occurred in the reactivating power of different sera or in the complement-content but even this variation was not marked, provided the person was in good health. Usually when 1/20 c.c. of unheated serum was added to 1 c.c. of heated serum, reactivation was complete for typhoid bacilli in twenty-four hours, and this mixture would become as destructive as 1 c.c. of fresh unheated serum. *B. coli*, on the other hand, proved much more resistant, so that it required 6/10 c.c. of fresh unheated serum to reactivate 1 c.c. of heated serum (Table I.).

TABLE I.[1]

Observation I. W. T. L., aged 25 years. No typhoid. Healthy.
Blood drawn June 11, 4 p.m. Serum used June 12, 3.30 p.m.

	Control	Immediate	1 hour	5 hours	24 hours
1 c.c. of unheated serum + B. coli	38,000	6,820	3,180	2	Sterile
1 c.c. ,, ,, + B. typhi	45,000	10,000	5,350	15	,,
1 c.c. of heated serum + 1/20 c.c. un-heated serum + B. typhi	45,000	9,950	5,180	270	,,
1 c.c. of heated serum + 6/10 c.c. un-heated serum + B. coli	38,000	4,900	2,600	3	,,

Observation II. M. G., aged 23 years. No typhoid. Healthy.
Blood drawn June 18, 5 p.m. Serum used June 19, 3.30 p.m.

	Control	Immediate	1 hour	5 hours	24 hours
1 c.c. unheated serum + B. coli	36,000	5,500	3,200	4	Sterile
1 c.c. ,, ,, + B. typhi	24,000	5,520	1,150	Sterile	,,
1 c.c. heated serum + 1/20 c.c. un-heated serum + B. typhi	24,000	4,740	2,920	110	,,
1 c.c. heated serum + 6/10 c.c. un-heated serum + B. coli	36,000	4,140	3,000	3	,,

[1] For brevity's sake *B. typhosus* is styled *B. typhi* in the tables.

It sometimes happened that when blood was used from an individual whose health was not up to the standard, bacteriolysis was not so rapid as in the above cases. This slight difference was well brought out in one instance where two observations were made upon the blood of the same person—the first one during the spring months, the second after a month's vacation. The first examination is shown in Observation I. In the second examination bacteriolysis was much more rapid than in the first, and when 1/20 c.c. of fresh serum was added to 1 c.c. of heated serum, great numbers of typhoid bacilli were completely destroyed in five hours (Table II.).

TABLE II.

Observation III. W. T. L.

Blood drawn September 21, 2.30 p.m. Serum used September 22, 12 m.

	Control	Immediate	1 hour	5 hours	24 hours
1 c.c. heated serum + 1/20 c.c. un-heated serum + B. typhi	Innumer-able	10,000	3,200	Sterile	Sterile
1 c.c. heated serum + 6/10 c.c. un-heated serum + B. coli	10,000	3,100	1,480	46	,,

In still another case—a middle-aged man who used alcohol in excess—the reactivating power of the blood was much below the normal average. This observation is directly in accord with the work of Abbott and Bergey. These slight differences in the reactivating power of normal sera, are, of course, dependent upon the complement-content of the blood. If an individual's health is slightly impaired the complement-content of his serum falls only a little below normal; but if an actual injury is done to his tissues, as in the constant use of alcohol, the decrease would seem to be more pronounced.

Finally, the interactivating power of different normal sera was tried; that is, the complement[1] of one normal serum was added to the intermediary body of another. In several cases it appeared to make little or no difference whether complement A was added to intermediary body B or complement A to intermediary body A. Table III.

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[1] The designation "complement" is not wholly accurate, as the full normal serum was employed. The quantity used is, however, active through its complements wholly or in large part.

TABLE III.

Observation IV.   A. Healthy male.   *B.* Healthy male.

Serum A.   Blood drawn May 15, 6 p.m.
Serum B.   Blood drawn May 23, 4 p.m.   Serum used May 24, 1 p.m.

	Control	Immediate	1 hour	5 hours	24 hours
1 c.c. *A*'s heated serum + 1/20 c.c. *B*'s unheated serum + B. typhi	50,000	9,800	5,080	8	Sterile
1 c.c. *A*'s heated serum + 7/10 c.c. *B*'s unheated serum + B. coli	39,000	2,600	1,300	Sterile	,,

From the results of the examination of normal sera it is evident that for the two strains of bacteria used 1 c.c. of unheated serum will destroy large numbers of both colon and typhoid bacilli in twenty-four hours, the reduction in numbers being more rapid with typhoid than with colon bacilli; and that 6/10 c.c. of unheated serum will reactivate 1 c.c. of heated serum for *B. coli*, while 1/20 c.c. of unheated serum will reactivate 1 c.c. of heated serum for *B. typhosus*.   With these figures as a standard for the complement-content of healthy normal serum, the following experiments were made upon the bacteriolytic power of the blood of 17 different individuals suffering from nephritis, heart disease, and other chronic affections.

The 17 patients can be summarized as follows: 11 cases of uraemia, with nine deaths; two cases of cirrhosis of the liver, with one death; one fatal case of pericarditis; one fatal case of aortic aneurism; one case of chronic endocarditis, living; and one case of diabetes mellitus, which has been lost sight of.   Autopsies were made in four of the 12 fatal cases.   Blood cultures were also made in four instances, and in several cases at least two observations were made upon the patient's serum.   All these cases can be roughly divided into three groups:

Group I.   Bacteriolytic action of the serum much diminished; reactivation difficult; 10 cases, seven deaths.

Group II.   Bacteriolytic action of serum very slightly reduced; reactivation fairly good; five cases, two deaths.

Group III.   No reduction in bacteriolytic power of serum; reactivation complete; two cases, two deaths.

Group I.

In Group I. there are six cases of uraemia, all but one of which died; one fatal case of cirrhosis of the liver; one fatal case of aortic aneurism; one case of diabetes mellitus and one case of chronic endocarditis, both living. Autopsies were obtained in four of the seven fatal cases. With the exception of the case of cirrhosis of the liver, the blood was usually drawn two to four days before death; and when there were two examinations of the blood they were made at intervals of two to three days, the last a few hours before the patient died.

If the bacteriolytic power of the serum from the above chronic cases is compared with the action of normal serum, the most striking difference is immediately apparent. Whereas a rapid reduction in the number of colon and typhoid bacilli, with total destruction of all bacteria took place with 1 c.c. of unheated normal serum in twenty-four hours, 1 c.c. of unheated serum from these cases produced little or no effect upon the colon bacilli, and sometimes did not even suffice to destroy typhoid bacilli in twenty-four hours. *B. coli* is fairly resistant even to the action of normal serum, so that it affords a very delicate test for the detection of slight reductions in bacteriolysis; but since heated abnormal[1] serum would almost never be successfully reactivated against this organism, it offers no means by which an estimation of the actual reduction in bacteriolysis could be measured. Fortunately, the typhoid bacillus is so easily destroyed that it was exceedingly rare to find serum of a case in which 1 c.c. did not produce a fairly marked bacteriolytic effect upon it. Moreover, reactivation could usually be accomplished, and thus by comparing with normal serum the amount of unheated abnormal serum required to reactivate 1 c.c. of heated serum a definite ratio between the two and scale of measure could be readily obtained. By this means it was estimated that reactivation was three to six times as difficult with the serum from this group of chronic cases as with normal serum; in other words instead of requiring 1/20 c.c. of fresh serum, 1/10, 2/10, or even 3/10 were necessary for complete bacteriolysis in twenty-four hours (Table IV.).

After it was found that a great reduction in the reactivating properties of these chronic sera existed, the question arose as to the exact cause of this change. Three possibilities immediately present themselves: first, that there is a reduction in the intermediary body of

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[1] This term will be used to designate the serum obtained from the diseased individuals.

TABLE IV.

Observation V. J. C., male, aged 64 years? Uraemia. Death May 10, one day after blood was drawn.

Blood drawn May 2, 3 p.m. Serum used May 10, 2 p.m. Autopsy: Chronic nephritis; general Streptococcus infection.

	Control	Immediate	1 hour	5 hours	24 hours
1 c.c. unheated serum + B. coli	39,000	10,740	8,040	2,100	Innumerable
1 c.c. ,, ,, + B. typhi	50,000	12,400	1,760	15	Sterile
1 c.c. heated serum + 1/20 c.c. } unheated serum + B. typhi	50,000	16,000	15,000	14,080	Innumerable
1 c.c. heated serum + 1/10 c.c. } unheated serum + B. typhi	50,000	10,120	10,920	6,740	,,
1 c.c. heated serum + 2/10 c.c. } unheated serum + B. typhi	50,000	14,000	11,400	960	42
1 c.c. heated serum + 8/10 c.c. } unheated serum + B. coli	39,000	5,860	4,860	3,130	Innumerable

Observation VI. M., male, aged 60 years. Uraemia; death April 18. No autopsy.

A. Blood drawn April 17, 5 p.m. Serum used 3.30 p.m. April 18 = Serum A. Healthy serum = serum B.

1 c.c. unheated serum A + B. coli	44,750	7,240	7,580	29	2
1 c.c. ,, ,, A + B. typhi	50,000	13,300	7,120	6,450	120
1 c.c. heated serum A + 1/20 c.c. } unheated serum A + B. typhi	50,000	11,325	8,000	10,000	Innumerable
1 c.c. heated serum A + 1/10 c.c. } unheated serum A + B. typhi	50,000	11,460	7,150	7,900	,,
1 c.c. heated serum B + 1/10 c.c. } unheated serum A + B. typhi	50,000	12,250	7,080	11,450	,,
1 c.c. heated serum B + 2/10 c.c. } unheated serum A + B. typhi	50,000	10,000	7,340	6,120	,,
1 c.c. heated serum A + 8/10 c.c. } unheated serum A + B. coli	44,750	6,480	3,380	620	,,

Observation VII. M. C., female, aged 62 years. Chronic alcoholism; uraemia; recovery.

Blood drawn April 9, 4.30 p.m. Serum used April 10, 1 p.m. = Serum C. Normal serum = serum B.

1 c.c. unheated serum C + B. coli	Innumerable	16,800	4,460	4	Sterile
1 c.c. ,, ,, C + B. typhi	,,	30,700	8,980	17	,,
1 c.c. heated serum C + 1/10 c.c. } unheated serum C + B. typhi	,,	10,900	6,440	16,500	Innumerable
1 c.c. heated serum B + 1/10 c.c. } unheated serum C + B. typhi	,,	14,000	7,440	1,010	,,
1 c.c. heated serum B + 2/10 c.c. } unheated serum C + B. typhi	,,	13,200	13,600	584	,,
1 c.c. heated serum C + 7/10 c.c. } unheated serum C + B. coli	,,	18,500	13,600	9,700	,,

Observation VIII. D. P., male, aged 45 years. Clinical diagnosis: Aneurism.

Blood drawn January 8, 8 p.m. Serum used January 9, 11 a.m. Blood cultures. Four c.c. of blood in three bouillon flasks of 250 c.c. Cultures sterile.

1 c.c. unheated serum + B. coli	38,000	5,000	4,980	6	Innumerable
1 c.c. ,, ,, + B. typhi	Innumerable	44,900	3,550	122	,,

Death January 11? Two days before death signs of consolidation at left base. Diagnosis: Pneumonia.

the serum : second, that there is a formation of auto-anticomplement ; and third, that there is a true reduction in complement.

The serum from a case which showed decided reduction in bacteriolysis was heated, and to 1 c.c. of this heated abnormal serum small quantities of fresh complement-containing serum from a normal patient were added; reactivation was complete. For typhoid bacilli, 1/20 c.c. of fresh normal serum restored the bacteriolytic property of 1 c.c. of the heated abnormal serum, and 6/10 c.c. of fresh abnormal serum did the same for the colon bacilli (Table V.).

TABLE V.

Observation IX.   Serum from Observation I. = serum D.   Uraemia; death.

Normal serum = serum E.   Healthy male, aged 28 years.   Blood drawn May 15, 6 p.m. Serum used May 16, 12 m.

	Control	Immediate	1 hour	5 hours	24 hours	48 hours
1 c.c. heated serum D + 1/10 c.c. unheated serum D + B. typhi	50,000	10,120	10,920	6,740	Innumerable	
1 c.c. heated serum D + 8/10 c.c. unheated serum D + B. coli	39,000	5,860	4,860	3,160	,,	
1 c.c. heated serum D + 1/20 c.c. unheated serum E + B. typhi	23,000	3,580	4,500	1,700	45	Sterile
1 c.c. heated serum D + 6/10 c.c. unheated serum E + B. coli	37,000	4,040	2,180	4	Sterile	,,

TABLE VI.

Observation X.   Abnormal serum = serum M.   Uraemia; death.

Normal serum = serum N.

	Control	Immediate	1 hour	5 hours	24 hours
1 c.c. heated serum M + 1/10 c.c. unheated serum M + B. typhi	50,000	11,460	7,150	7,900	Innumerable
1 c.c. heated serum M + 8/10 c.c. unheated serum M + B. coli	44,750	7,420	6,040	2,160	,,
1 c.c. heated serum N + 1/10 c.c. unheated serum M + B. typhi	50,000	12,250	7,080	11,450	,,
1 c.c. heated serum N + 8/10 c.c. unheated serum M + B. coli	44,750	6,480	3,380	620	,,

Observation XI.   Abnormal serum = serum O.   Uraemia; death.

Healthy serum = serum E.   Same as serum E in Observation VIII.   Table IV.

	Control	Immediate	1 hour	5 hours	24 hours
1 c.c. heated serum O + 7/10 c.c. unheated serum O + B. coli	60,000	3,720	3,950	2	Innumerable
1 c.c. heated serum E + 7/10 c.c. unheated serum O + B. coli	60,000	4,200	3,700	960	,,

A second experiment was made. Serum from a normal patient was heated, and fresh abnormal serum from cases of uraemia, etc., added in small quantities. There was no reactivation, or rather, fresh abnormal serum M or O did not reactivate heated normal serum N or E any better than its own serum (Table VI.).

Fresh normal serum, then, will reactivate serum which has partially lost its bacteriolytic properties; but this latter abnormal serum will not reactivate heated normal serum. There can be no reduction in the intermediary body, nor can a production of auto-anticomplement account for the entire diminution in bacteriolysis. Anticomplement is stable, and is known to remain uninjured even when heated to 60° C. (London)[1]; it would, therefore, be present in the heated serum, and prevent the action of the complement contained in the added fresh normal serum—that is, if auto-anticomplement existed, the complement of fresh normal serum would probably be neutralized, and hence it would fail to reactivate the heated abnormal serum. There can be only one conclusion: the entire phenomenon is due to an actual decrease in complement. The reduction in bacteriolysis and the imperfection of reactivation are due to a diminution in complement.

The question of the relation of this diminution in complement to the development of terminal infections is important. Do those cases which show the most marked change in the complement really succumb to infection?

In each of the four autopsies a definite infection could be discovered (Table VII.).

Table VII.

Observation XII. D. D., male, aged 45 years. Clinical diagnosis; uraemia: pneumonia? Blood drawn February 10, 4.30 p.m. Serum used February 11, 10.30 a.m.

	Control	Immediate	1 hour	5 hours	24 hours
1 c.c. unheated serum + B. coli	Innumer- able	20,300	3,950	—	455
1 c.c. ,, ,, + B. typhi	46,800	14,100	4,900	—	350
1 c.c. heated serum + 1/10 c.c. } unheated serum + B. typhi }	Innumer- able	26,500	12,500	—	19

Death February 10, 11.30 p.m. Autopsy 196.

Anatomical diagnosis: Chronic interstitial nephritis; multiple infarctions of lung.

[1] London. *Centralbl. f. Bakteriol.*, 1902, xxxii. p. 48, p. 147.

Observation XIII. A., female, aged 55 years. Clinical diagnosis : cirrhosis of liver.

Blood drawn February 19, 4.30 p.m. Serum used February 20, 1.30 p.m.

1 c.c. unheated serum + B. coli	43,000	3,750	3,930	820	Innumerable
1 c.c. „ „ + B. typhi	50,000	3,840	2,160	870	Sterile
1 c.c. heated serum + 1/10 c.c. unheated serum + B. typhi	50,000	4,890	3,180	3,160	5
1 c.c. heated serum + 4/10 c.c. unheated serum + B. typhi	50,000	3,740	1,920	223	Sterile

Death April 17. Anatomical diagnosis : Atrophic cirrhosis of liver ; general infection with *B. coli.*

Observation XIV. D. P., male, aged 30 years. Clinical diagnosis : Uraemia.

Blood drawn March 22, 10 a.m. Serum used March 23, 11 a.m.

1 c.c. unheated serum + B. coli	176	80	29	74	Increase
1 c.c. „ „ + B. typhi	9,500	2,820	888	7	Sterile
1 c.c. heated serum + 1/20 c.c. unheated serum + B. typhi	9,500	3,140	1,080	39	„
1 c.c. heated serum + 1/10 c.c. unheated serum + B. typhi	9,500	2,650	1,060	18	1
1 c.c. heated serum + 2/10 c.c. unheated serum + B. typhi	9,500	2,360	640	12	Sterile
1 c.c. heated serum + 8/10 c.c. unheated serum + B. coli	176	28	21	58	Innumerable

Death March 24. Anatomical diagnosis : Chronic interstitial nephritis, acute bronchopneumonia.

Observation XV. J. C., male, aged 64 years ? Uraemia.

Blood drawn May 6, 3.30 p.m. Serum used May 7, 12 m.

1 c.c. unheated serum + B. coli	40,000	59,000	13	380	Innumerable
1 c.c. „ „ + B. typhi	50,000	11,780	4,660	11	Sterile
1 c.c. heated serum + 1/20 c.c. unheated serum + B. typhi	50,000	11,780	10,270	2,800	2
1 c.c. heated serum + 1/10 c.c. unheated serum + B. typhi	50,000	13,450	4,020	2	Sterile
1 c.c. heated serum + 8/10 c.c. unheated serum + B. coli	40,000	4,400	3,940	730	Innumerable

May 9. Patient much worse. Blood cultures = Streptococcus pyogenes.

Blood drawn May 9, 3 p.m. Serum used May 10, 2.30 p.m.

1 c.c. unheated serum + B. coli	39,000	10,740	8,040	2,100	Innumerable
1 c.c. „ „ + B. typhi	50,000	12,400	1,760	15	Sterile
1 c.c. heated serum + 1/20 c.c. unheated serum + B. typhi	50,000	16,000	15,000	14,080	Innumerable
1 c.c. heated serum + 1/10 c.c. unheated serum + B. typhi	50,000	10,120	10,920	6,740	„
1 c.c. heated serum + 2/10 c.c. unheated serum + B. typhi	50,000	14,000	11,400	960	42
1 c.c. heated serum + 8/10 c.c. unheated serum + B. coli	39,000	5,860	4,860	3,130	Innumerable

Death May 10. Anatomical diagnosis : General arterial sclerosis, chronic interstitial nephritis, terminal pneumonia, general Streptococcus infection.

Case XV. is of special interest. The first examination showed only a mild complement decrease, but two days later the complement had fallen perceptibly, and *Streptococci* were recovered from the blood. Certainly, the development of the infection is perfectly clear—the gradual reduction of complement with consequent crippling of the protective power of the serum, the onset of an infection, an overwhelming multiplication of bacteria, and then death from general streptococcus infection.

In two other fatal cases of uraemia, which, however, did not come to autopsy, a terminal infection of some kind was suspected from a marked hyperleucocytosis and a rise in temperature; and in a fatal case of aneurism signs of pneumonia were found a few days before death.

The following result obtained in the case of diabetes mellitus needs no comment, since secondary infections in this disease are notoriously common (Table VIII.).

TABLE VIII.

Observation XVI.   Philadelphia Hospital.   Female, aged 60 years ?   Clinical diagnosis: diabetes mellitus.

	Control	Immediate	1 hour	5 hours	24 hours
1 c.c. unheated serum + B. coli	20,900	5,000	2,500	143	Innumerable
1 c.c. ,, ,, + B. typhi	20,200	10,000	5,200	394	4,400
1 c.c. heated serum + 1/10 c.c.) unheated serum + B. typhi)	20,200	6,300	5,900	184	1,500

Finally, the two cases of uraemia with recovery must be considered. These illustrate the possibility of a decrease in complement, perhaps fleeting, without infection and with subsequent improvement. In these instances the temporary disappearance of the complement, while exposing the individual to infection, may be of short duration, in which event the danger may be accidentally escaped, or infection of light degree occurring, the restitution of complement may suffice to overcome it. But it must be borne in mind that, after all, the use of two organisms which are not the usual ones of terminal infection is only an indirect mode of establishing total bacteriolytic complement-content. Where so many complements are dealt with it might readily happen that the conditions for *Streptococci* and *Staphylococci*, the chief agents of terminal infection, might be quite different. The latter organisms do not, unfortunately, lend themselves minutely to discriminating tests.

The results of examinations from the 10 cases which comprise this group are undoubtedly very suggestive. Every case showed a pronounced decrease in the complement-content of the blood, and the mortality among them was 70 per cent. Of the three patients who did not die, one had diabetes mellitus and one chronic alcoholism. Terminal infection was proven to be the immediate cause of death in five cases, and was suspected to have existed in the other two cases.

Group II.

This group includes a series of records much less convincing than those of Group I. Of the five cases only two are known to have died, and neither blood cultures nor autopsies were obtained in any case; one case of uraemia left the hospital improved, and a fourth case was lost sight of; the fifth case, still living, is one of cirrhosis of the liver. In most of the cases bacteriolysis did not take place with normal amounts of reactivating serum; in the rest, bacteriolysis was simply prolonged. Bacteriolysis, however, in every instance was distinctly below normal. The mortality was 40 per cent. (Table IX.).

Table IX.

Observation XVII. A. F., male, aged 53 years. Clinical diagnosis: Cirrhosis of liver; patient living.

Blood drawn February 19, 6 p.m. Serum used February 20, 12 m.

	Control	Immediate	1 hour	5 hours	24 hours
1 c.c. unheated serum + B. coli	43,000	5,300	3,260	8	Sterile
1 c.c. „ „ + B. typhi	50,000	5,520	635	1	„
1 c.c. heated serum + 1/10 c.c. unheated serum + B. typhi	50,000	6,320	4,530	1,900	32
1 c.c. heated serum + 2/10 c.c. unheated serum + B. typhi	50,000	3,660	1,690	310	Sterile

Observation XVIII. Philadelphia Hospital. Male, aged 70 years? Uraemia; unconscious.

Blood drawn February 26, 4.30 p.m. Serum used February 27, 11 a.m.

	Control	Immediate	1 hour	5 hours	24 hours
1 c.c. unheated serum + B. coli	42,100	9,650	11,600	416	Sterile
1 c.c. „ „ + B. typhi	Innumerable	15,100	10,200	412	„
1 c.c. heated serum + 1/20 c.c. unheated serum + B. typhi	„	13,500	10,500	11,600	424
1 c.c. heated serum + 1/10 c.c. unheated serum + B. typhi	„	11,425	12,000	5,400	87

Indeed, these records probably represent the usual state of the blood in such maladies as nephritis or cirrhosis of the liver, and suggest

that it is not until the most critical stages of the disease are reached that a great reduction in the complement-content of the blood takes place.

Group III.

Finally, we come to this last group of cases, comparatively small, it is true, but none the less important. In two fatal cases the blood showed no change from the normal as regards its complement-content. One patient died of uraemia; the other, a man, aged thirty years, of pericarditis with effusion. No autopsy is recorded in the second case. The first patient developed symptoms of uraemia after a long attack of chronic nephritis, and remained unconscious for several days. During this time two examinations were made of her blood—the first, three days before death; the second, one day later. At the time of the second examination blood cultures were obtained. No growth developed in any of the bouillon flasks (Table X.).

Table X.

Observation XIX. S. T., female, aged 58 years? Clinical diagnosis : Uraemia.

Blood drawn June 24, 2.30 p.m. Serum used June 25, 10.30 a.m. June 24, leucocytes, 26,150.

	Control	Immediate	1 hour	5 hours	24 hours
1 c.c. unheated serum + B. coli	20,900	6,800	3,750	7	Sterile
1 c.c. ,, ,, + B. typhi	20,200	6,200	2,300	1	,,
1 c.c. heated serum + 1/20 c.c. } unheated serum + B. typhi	20,200	7,300	1,300	5	,,
1 c.c. heated serum + 6/10 c.c. } unheated serum + B. coli	20,900	4,600	1,300	6	12,500
1 c.c. heated serum + 8/10 c.c. } unheated serum + B. coli	20,900	3,800	1,480	4	Sterile

June 25. Patient about the same. Has had salt infusions. Blood cultures = sterile.

Blood drawn June 25, 3 p.m. Serum used June 26, 2.30 p.m. Leucocytes, 21,900.

	Control	Immediate	1 hour	5 hours	24 hours
1 c.c. unheated serum + B. coli	16,300	3,400	674	10	Sterile
1 c.c. ,, ,, + B. typhi	20,600	5,950	488	Sterile	,,
1 c.c. heated serum + 1/20 c.c. } unheated serum + B. typhi	20,600	5,800	4,200	60	,,
1 c.c. heated serum + 6/10 c.c. } unheated serum + B. coli	16,300	3,200	936	3	1
1 c.c. heated serum + 8/10 c.c. } unheated serum + B. coli	16,300	956	346	46	Sterile

Death, June 27.

The patient died two days after the last specimen of blood was taken. The anatomical diagnosis at autopsy was chronic interstitial

nephritis, hypertrophy and dilatation of the heart, œdema and congestion of the lungs. Cultures from the heart's blood, spleen, liver, and kidneys gave negative results. On plates from the lungs a few colonies of *Streptococcus pyogenes* were found.

Here, then, is a case without reduction of complement and without demonstrable terminal infection. As far as bacteriological methods go, neither before nor after death was an infection of any kind revealed. Certainly, this case furnishes a supplement for the cases of Group I. We know it is not every case of Bright's disease or heart disease that dies from a terminal infection, nor do all these cases show a reduction in complement; and the mere knowledge of these facts cannot but suggest that there must be some sort of relationship between the two. That in this instance a high complement-content should be associated with the absence of demonstrable terminal infection strongly suggests that one condition is dependent upon the other, and that without complement reduction there is no terminal infection. To support this view still further we can only advance the many positive cases where the complement was reduced and terminal infection did develop.

When the results of this series of experiments are summed up they seem to show that in general during the course of a prolonged chronic disease, such as nephritis, cirrhosis of the liver, and heart disease, the bacteriolytic complement-content of the blood usually falls below normal. If the patient gets seriously worse, the reduction becomes so great that the resistance of the patient is dangerously injured, and the body is no longer protected against the entrance of bacteria. At this time the slightest exposure affords ample opportunity for the entrance of bacteria into the body; and this entrance once effected, an infection is directly set up, which threatens rapidly to terminate fatally. If the necessary exposure is in any way prevented, and the individual tides over this critical period of greatly decreased complement, or has perhaps only a slight decrease in complement, the chances of recovery are good. Just this state of affairs is exemplified in a severe case of cardiac break-down, and such a case is illustrated in Group I.

Finally, a small percentage of cases do not show a diminution in the complement-content of their blood, and it is in all likelihood these cases that escape terminal infection.

The complement may be looked upon as an index to the resistance of the patient. If the complement is decreased, the resistance is lowered; if the complement remains above normal limits, the resistance is

high.   But it must not be forgotten that even the normal is not an absolutely fixed quantity ; very slight fluctuations are found in comparatively healthy persons, and according to the well-being or ill-being of that person the complement rises or falls.

After the above results were obtained a second series of experiments was undertaken to see whether a decrease in the complement-content of the blood could explain the development of general infections following localized wound infections.   It was exceedingly difficult to find suitable cases for this purpose, and the number of observations is, therefore, small.   In only one case, moreover, was it possible to prove that a wide-spread infection existed.   This was a fatal case of peritonitis. Most of the cases were fairly mild local infections, with rapid recovery. They include nine observations, all of which can be roughly divided into two main groups.   In the first the complement was increased, in the second it was decreased.

Group I. comprises :

Cellulitis of arm .... 1 case.	Recovery.
Appendicitis .... 1 ,,	,,
Cellulitis of leg .... 1 ,,	,,
Pyonephrosis........................ 1 ,,	Death.
Infected scalp wound............ 1 ,,	Recovery.
Pelvic abscess .... 1 ,,	,,
General peritonitis .... 1 ,,	Death.

When the blood was drawn for examination the patient's white blood corpuscles were always counted.   In every case there was a hyperleucocytosis ranging from 13,000 to 25,000.   The serum of every case, even of those that terminated fatally, showed an increase in the complement for *B. coli* and *B. typhosus*.   Sometimes with *B. coli* only one-third the normal amount of complement was necessary to reactivate 1 c.c. of heated serum (Table XI.).

The association of the rise in complement with the hyperleucocytosis is noteworthy, and appears to agree with the results of those observers who consider that the complement or alexin takes its origin or at least is present in the bodies of the polymorphonuclear leucocytes.   Wassermann[1] has reviewed this subject, and has himself been able to produce an anticomplement for the serum of rabbits by injecting rabbit's washed leucocytes into the peritoneal cavity of guinea-pigs; but anticomplement was obtained in such small quantity that he believes, though the poly-

[1] Wassermann.   *Zeitschrift f. Hygiene u. Infektionskrankheiten*, 1901, xxxvii. p. 173.

morphonuclear leucocytes undoubtedly contain a certain amount of complement, they cannot give rise to its total in the body.

TABLE XI.

Observation XX. J. T., male, aged? Clinical diagnosis: Cystitis. July 17, leucocytes, 17,100.

Blood drawn July 17, 8 p.m. Serum used July 18, 1.30 p.m.

	Control	Immediate	1 hour	5 hours	24 hours
1 c.c. unheated serum + B. coli	14,500	8,900	2,200	Sterile	Sterile
1 c.c. „ „ + B. typhi	22,000	16,400	1,300	,,	,,
1 c.c. heated serum + 1/20 c.c. ⟩ unheated serum + B. typhi ⟨	22,000	17,700	2,000	,,	,,
1 c.c. heated serum + 6/10 c.c. ⟩ unheated serum + B. coli ⟨	14,500	2,080	800	,,	,,

Observation XXI. J. C., male, aged 35 years? Appendicitis: operation; general peritonitis.

Blood drawn July 21, 9 p.m. Serum used July 22, 11.30 a.m. July 21, leucocytes, 29,400.

1 c.c. unheated serum + B. coli	Innumer- able	12,900	700	Sterile	Sterile
1 c.c. „ „ + B. typhi	29,000	7,700	146	,,	,,
1 c.c. heated serum + 1/20 c.c. ⟩ unheated serum + B. typhi ⟨	29,000	8,670	2,800	106	,,
1 c.c. heated serum + 4/10 c.c. ⟩ unheated serum + B. coli ⟨	Innumer- able	6,800	2,900	Sterile	,,

Death, July 23. Autopsy. Anatomical diagnosis: acute general fibrinopurulent peritonitis.

Observation XXII. P., male, aged 58 years. Infected scalp wound. June 30, leucocytes, 13,900.

Blood drawn June 30, 12.30 p.m. Serum used June 31, 12.30 p.m. Recovery.

1 c.c. unheated serum + B. coli	Innumer- able	4,400	830	Sterile	Sterile
1 c.c. „ „ + B. typhi	,,	33,220	4,030	,,	,,
1 c.c. heated serum + 1/20 c.c. ⟩ unheated serum + B. typhi ⟨	,,	22,000	8,200	,,	,,
1 c.c. heated serum + 6/10 c.c. ⟩ unheated serum + B. coli ⟨	,,	4,900	2,800	,,	,,

Group II. In the second group there are only two cases—one of gangrene of the arm and one of carcinoma of the uterus, with operation and intestinal resection, followed by pelvic abscess and death.

The first patient (Table XII.) was exceedingly ill when the blood was taken, but unfortunately the subsequent history was not obtainable, so that the fate of the case is unknown.

Table XII.

Observation XXIII. M. T., male, aged 16 years? Clinical diagnosis: gangrene of arm; amputation.

Blood drawn July 26, 1 p.m. Serum used July 27, 10 a.m.

	Control	Immediate	1 hour	5 hours	24 hours	48 hours
1 c.c. unheated serum + B. coli	25,500	6,800	800	Sterile	Sterile	Sterile
1 c.c. „ „ + B. typhi	34,400	15,200	2,600	132	1	,,
1 c.c. heated serum + 1/20 c.c. unheated serum + B. typhi	34,400	14,400	4,200	12	15	Innumerable
1 c.c. heated serum + 1/10 c.c. unheated serum + B. typhi	34,400	12,500	3,200	6	Sterile	Sterile
1 c.c. heated serum + 6/10 c.c. unheated serum + B. coli	25,500	3,200	294	65	2,600	Innumerable

Table XIII.

Observation XXIV. M. K., female, aged 65 years? Carcinoma of uterus. Operation. Resection of descending colon. July 4, leucocytes 26,600.

Blood drawn July 4, 10 a.m. Serum used July 5, 12 m.

	Control	Immediate	1 hour	5 hours	24 hours
1 c.c. unheated serum + B. coli	29,700	8,000	2,000	8	Sterile
1 c.c. „ „ + B. typhi	19,100	7,700	4	Sterile	,,
1 c.c. heated serum + 1/20 c.c. unheated serum + B. typhi	19,100	6,600	242	2	,,
1 c.c. heated serum + 2/10 c.c. unheated serum + B. coli	29,700	6,500	3,500	1,000	,,

July 11. Patient doing fairly well. Leucocytes 20,300.

Blood drawn July 11, 3.30 p.m. Serum used July 12, 1 p.m.

	Control	Immediate	1 hour	5 hours	24 hours
1 c.c. unheated serum + B. coli	30,150	11,400	1,230	75	Sterile
1 c.c. „ „ + B. typhi	38,500	14,600	100	Sterile	,,
1 c.c. heated serum + 1/20 c.c. unheated serum + B. typhi	38,500	13,600	10,300	2,700	3
1 c.c. heated serum + 1/10 c.c. unheated serum + B. typhi	38,500	11,400	3,000	56	2
1 c.c. heated serum + 6/10 c.c. unheated serum + B. coli	30,150	5,900	4,800	1,010	45
1 c.c. heated serum + 8/10 c.c. unheated serum + B. coli	30,150	8,300	2,300	248	Sterile

From this time on the patient was much worse. Death, July 19. No autopsy.

The two observations in the second case (Table XIII.) are instructive, inasmuch as they show that a gradual reduction in the complement-content of the blood took place during the course of the patient's illness, corresponding to which the patient's condition grew worse. We were unable to make a third examination, and no autopsy was permitted, so

that the case remains somewhat incomplete, although the data are still sufficient to throw some light upon the cause of the unfavourable termination.

From this series of cases no very definite conclusions can be drawn other than that hyperleucocytosis has some influence upon the increase in complement for typhoid and colon bacilli. In only one of three fatal cases was there a decrease in complement, and even in this case the reduction must be looked upon as relative rather than real. It must be remembered, however, that with wound infections the danger of general invasion is from *Streptococci* or *Staphylococci*, and not from colon or typhoid bacilli, and the importance of this consideration was very definitely brought out in three or four observations upon the blood of typhoid fever patients.

It has already been shown by Ehrlich and Morgenroth[1], Wechsberg[2], Wendelstadt[3], and Marshall and Morgenroth[4] that haemolytic serum contains several specific haemolytic complements, and Wassermann[5] has succeeded in separating the general group of haemolytic complements from the bacteriolytic complements. The agglutinins, too, are known to be definite specific substances, and Verney[6] has furthermore demonstrated that if a given serum agglutinates both typhoid and colon bacilli, this property is due to the fact that the serum contains a specific agglutinin for *B. typhosus* and another for *B. coli*. Now since the general properties of haemolytic and bacteriolytic sera are so similar, it would not be surprising to find that bacteriolytic sera as well as haemolytic sera possessed not one common complement capable of affecting all bacteria, but a multitude of definite specific complements.

Typhoid fever represents a class of diseases in which the bacteria that cause the affection are widely distributed throughout the body. At one time during the course of the disease bacilli are almost always present in the circulating blood, and it is now fairly certain that typhoid fever is really a general infection characterized by local lesions, and not purely an infection of the intestinal tract.

For this reason it was thought that some interest might be attached to a study of the bacteriolytic properties of the blood of typhoid fever

[1] Ehrlich and Morgenroth. *Berliner klin. Wochenschrift*, 1900, No. 31.

[2] Wechsberg. *Wien. klin. Wochenschrift*, 1901, No. 48.

[3] Wendelstadt. *Centralbl. f. Bakteriol.*, 1902, xxxi. p. 469.

[4] Marshall and Morgenroth. *Centralbl. f. Bakteriol.*, 1902, xxxi. p. 570.

[5] Wassermann. *Op. cit.*

[6] Verney. *Centralbl. f. Bakteriol.*, 1902, xxxii. p. 290.

patients. In this connection the work of Richardson[1] may be cited. He found, using hanging-drop preparations, that typhoid sera were less destructive to typhoid bacilli than normal sera, and that the addition of normal sera to typhoid sera restored bacteriolysis.

TABLE XIV.

Observation XXV. W. D., male, aged 30 years. Typhoid fever, ninth day of disease. Death, Sept. 2.

Blood drawn Sept. 15, 4 p.m. Widal, negative, Sept. 15.

Serum used Sept. 16, 12 m. Blood cultures, Sept. 15: *B. typhosus.*

	Control	Immediate	1 hour	5 hours	24 hours
1 c.c. unheated serum + B. coli	40,000	11,000	1,300	27	Sterile
1 c.c. ,, ,, + B. typhi	50,000	16,500	960	3	,,
1 c.c. heated serum + 1/20 c.c. unheated serum + B. typhi	50,000	16,800	4,500	5,500	Innumerable
1 c.c. heated serum + 1/10 c.c. unheated serum + B. typhi	50,000	13,200	3,820	1,620	,,
1 c.c. heated serum + 2/10 c.c. unheated serum + B. typhi	50,000	15,600	2,120	38	36
1 c.c. heated serum + 6/10 c.c. unheated serum + B. coli	40,000	9,000	1,480	220	Sterile

Observation XXVI. W. S., aged 35 years. Typhoid septicaemia, acute endocarditis.

Blood drawn Sept. 23, 3 p.m. Widal, Sept. 23. Positive in 1 : 5000.

Serum used Sept. 24, 1 p.m.

Blood cultures Sept. 6: *B. typhosus.* Blood cultures Sept. 23, negative.

1 c.c. unheated serum + B. coli	60,000	14,800	940	3	Sterile
1 c.c. ,, ,, + B. typhi	50,000	17,800	8,200	33	,,
1 c.c. heated serum + 1/20 c.c. unheated serum + B. typhi	50,000	17,400	19,000	20,000	3,740
1 c.c. heated serum + 1/10 c.c. unheated serum + B. typhi	50,000	15,000	15,800	14,200	1,640
1 c.c. heated serum + 2/10 c.c. unheated serum + B. typhi	50,000	15,900	12,800	2,400	160
1 c.c. heated serum + 4/10 c.c. unheated serum + B. typhi	50,000	17,700	10,200	60	Sterile
1 c.c. heated serum + 6/10 c.c. unheated serum + B. coli	60,000	9,300	3,540	320	120

Observation XXVII. H. R., aged 28 years. Typhoid fever, mild. Widal, positive, Sept. 15.

Blood drawn Sept. 17, 4.30 p.m. Blood cultures, Sept. 17, negative.

Serum used Sept. 18, 11.30 a.m. Recovery.

1 c.c. unheated serum + B. coli	40,000	8,820	2,080	Sterile	Sterile
1 c.c. ,, ,, + B. typhi	50,000	10,840	3,160	44	,,
1 c.c. heated serum + 1/20 c.c. unheated serum + B. typhi	50,000	16,900	9,300	1,350	,,
1 c.c. heated serum + 6/10 c.c. unheated serum + B. coli	40,000	5,200	2,480	Sterile	,,

[1] Richardson. *Journal of Medical Research*, 1901, vi. p. 187.

In the present experiments the blood of three typhoid fever patients was examined. Case I. ended fatally; Case II. was probably a true case of typhoid septicaemia, with symptoms of acute endocarditis; and Case III. ran a mild, short course (Table XIV.).

In the first two cases typhoid bacilli were recovered from the circulating blood; in the third case blood cultures were negative, and the patient's temperature dropped to normal the day after blood was drawn. The significance of the above results, however, lies in the fact that the reactivating power of the serum was greatly diminished for typhoid bacilli and not for colon bacilli. *B. paracoli* (Cushing) and *B. dysenteriae* (Shiga) were next tried, and exactly the same result was obtained. The typhoid serum showed a decrease in reactivating power for typhoid bacilli, but for typhoid bacilli alone (Table XV.).

TABLE XV.

Observation XXVIII.   Healthy normal serum from Observation III.

	Control	Immediate	1 hour	5 hours	24 hours
1 c.c. heated serum + 1/10 c.c. un-heated serum + B. dysenteriae	40,000	9,700	2,260	Sterile	Sterile
1 c.c. heated serum + 2/10 c.c. un-heated serum + B. paracoli	60,000	9,500	270	,,	,,

Observation XXIX.   Typhoid serum from Case XXV.

1 c.c. heated serum + 1/10 c.c. un-heated serum + B. dysenteriae	19,000	4,040	1,840	1,940	Sterile
1 c.c. heated serum + 2/10 c.c. un-heated serum + B. paracoli	40,000	11,900	910	113	,,

If normal complement containing serum was now added to these typhoid sera, reactivation was complete (Table XVI.).

TABLE XVI.

Observation XXX.   Typhoid serum from Observation XXV. = P.   Normal serum from Observation III. = Q.

	Control	Immediate	1 hour	5 hours	24 hours
1 c.c. heated serum P + 1/20 c.c. unheated serum Q + B. typhi	Innumerable	8,800	7,600	2,580	Sterile
1 c.c. heated serum P + 6/10 c.c. unheated serum Q + B. coli	10,000	2,020	500	46	,,

This reduction in the specific bacteriolytic power of typhoid serum, evidenced by the difficulty in reactivating heated serum with unheated

typhoid serum for typhoid bacilli, is due solely to the fact that the complement for typhoid bacilli has been reduced—reduced for this organism, and for it alone[1]. It must be that in typhoid fever the specific typhoid complement is, as it were, picked out from the other bacteriolytic complements, and suffers a change to which *B. coli*, *B. dysenteriae*, and even *B. paracoli* are not subject. This means that we are dealing not with one common bacteriolytic complement, but with a multitude of specific complements, so highly differentiated that organisms as closely related as *B. typhosus* and *B. paracoli* can only be destroyed by the combination of their own peculiar complements with the corresponding intermediary body for that organism. In certain cases of typhoid fever the specific typhoid complement falls, and although the several complements for the other organisms remain unaltered, they can be of no assistance in the destruction of typhoid bacilli, inasmuch as they are unable to unite with the specific intermediary body for that bacillus.

Chronic diseases differ materially from acute diseases. In uraemia or heart disease probably all the complements suffer, though perhaps not in equal proportions; for Flexner[2] has shown that the bacteriolytic power of the blood in these cases is diminished toward the *Staphylococcus aureus*, and Laquer[3], Neisser and Doering[4], and Hedinger[5] have demonstrated a reduction of haemolysis in uraemia. But when *B. coli* and *B. typhosus* are alone used as indicators for the complement-content of serum, accurate results, even in chronic disease, cannot be hoped for in all cases, since it is the pyogenic cocci which are mostly concerned in the production of terminal infections; and in acute wound infections, where, probably as in typhoid fever, only a specific com-

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[1] After the completion of this article the important paper by Wright and Windsor (*Journal of Hygiene*, 1902, II. p. 385) appeared. The results given in it in respect to the bacilli of typhoid fever and Asiatic cholera bear directly upon a part, at least, of my studies. The preliminary diminution in bactericidal power of the blood which occurs in anti-typhoid inoculation is of interest in view of the reduction in complement for typhoid bacilli which takes place during the course of typhoid fever. A point in our results upon which we fail to agree entirely, but which a study directed especially to its elucidation might readily clear up, is the evidence in my studies upon normal serum of a difference between the bacteriolytic complements for typhoid and colon bacilli, Wright and Windsor having found that the bacteriolytic body of normal serum is one for both the bacilli of typhoid fever and Asiatic cholera. So far as the individuality of the complement for typhoid bacilli is concerned my results with normal serum are borne out by the tests upon the serum of typhoid patients in whom the complement for the colon bacillus and some other bacilli is unimpaired.

[2] Flexner. *Op. cit.*   [3] Laquer. *Op. cit.*
[4] Neisser and Doering. *Op. cit.*   [5] Hedinger. *Op. cit.*

plement or group of complements is affected, and those for the pyogenic cocci alone, satisfactory experiments cannot be made unless these bacteria are used as indicators for the complement-content of the blood, a procedure not as yet made practicable.

Conclusions.

1. Normal individuals show slight fluctuations in the bacteriolytic complement-content of their blood.

2. In many prolonged chronic affections, such as nephritis, cirrhosis of the liver, and diabetes mellitus, there is a marked decrease in the bacteriolytic blood complement, which becomes more marked towards the end of the disease.

3. Terminal infection in chronic disease is probably the direct result of the diminished state of the bacteriolytic complement.

4. The blood serum of certain individuals suffering from chronic disease does not show a reduction in complement; these individuals appear to escape terminal infection.

5. Hyperleucocytosis is frequently associated with high complement-content of the blood serum for typhoid and colon bacilli.

6. The blood serum of some typhoid fever patients shows a diminution in the specific complement for the typhoid bacillus.

7. Human blood serum contains a multiplicity of bacteriolytic complements.

ON SOME FACTORS IN BACTERIOLYTIC ACTION[1].

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THAT blood or serum and other body fluids, such as the natural or artificially excited exudations, possess bacteriolytic power is a familiar observation.

As the result of a series of experiments carried out on guinea-pigs in Berne early last year (1901) I was able to publish[2] evidence confirmatory of the fact originally observed by Nuttall[3] nearly 15 years ago, that the supposed ferment (complement, addiment) upon which this power depends suffers a rapid disappearance from the fluids in question after their removal from the living body. The experiments also showed that a complement available for guinea-pigs and at the same time satisfactory to the immune body which was used for their protection—that of horses immunised against the *Bacillus typhosus*—was supplied by the fresh serum of all the species of animals examined (ox, sheep and pig), and therefore is not so special to the species as was originally held by Professor Ehrlich. And this complement I found to exist not only in the serum, but also in fresh clot from which the serum had been separated; a fact which lends support to the contention that the bacteriolytic ferment is of leucocytic origin.

These observations have now been followed up in an examination of the bacteriolytic power of blood and serum in the test-tube; more

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[1] A preliminary note of this paper was published in *The Lancet* of Jan. 4th, 1902, p. 18, entitled "The Disappearance of the Addiment from Anti-Microbic Sera." Its publication in full has been unavoidably delayed. (MS. received 4. XII. 1902. *Ed.*)

[2] *Journal of Hygiene*, 1902, Vol. II. p. 85, "On the Protective Substances of Immune Sera."

[3] *Zeitschr. f. Hygiene*, 1888, Bd. IV. pp. 353–394.

especially with reference to the time relations which are found to exist, and the quantitative influence exerted by the immune body upon the process of bacteriolysis.

Methods.

The plan of the experiments was arranged as follows. A number of rabbits were immunised by the successive injection of increasing doses of living typhoid culture, and were subsequently bled for the preparation of their sera. The weights and temperatures of the animals were recorded daily. Three of the animals instead of rapidly regaining the temporary loss of weight which followed each injection of typhoid bacilli became distinctly ill with loss of appetite, languor and wasting. They were intentionally bled and killed at an unfavourable period in the immunity reaction in order to determine to what extent the bacteriolytic action of their sera was affected by their condition.

The immunisation of the rabbits is shown in Table I. below. The animal experiments were done in Berne last year (1901) in the laboratory of Professor Tavel, to whom I was again indebted for the opportunity of working there.

The sera yielded by the rabbits were examined for bacteriolytic action on broth cultures of the *Bacillus typhosus* of a definite age, and were compared in this respect with the fresh sera of five normal rabbits. The following procedure was employed. The blood was taken by means of a sharp-pointed metal cannula from the exposed femoral artery[1], and was received into sterile test-tubes and allowed to clot, the clot being subsequently loosened a little from the side of the tube by means of a stout platinum needle in order to assist the separation of the serum.

So soon as fluid began to separate from the clot a specimen was removed for examination, and after an interval of from four to six hours from the time of bleeding a second specimen was taken. At the end of 24 hours the remainder of the serum was removed and placed in sterile tubes for later use.

Anti-bacterial action was tested as follows: the serum to be examined was measured out by means of a sterilised pipette from which a given number of drops were allowed to fall into a sterile test-tube. The same pipette was always used throughout each series of

[1] The insertion of the cannula requires a little dexterity, but the method has the advantage of ensuring sterility and yielding a relatively large amount of blood.

Table I.

Immunisation of Rabbits.

Rabbit	Day 1	Day 3	Day 5	Day 10	Day 14	Day 19	Day 21
I. a.	$\frac{1}{10}$th of a 24-hours old agar culture subcutaneously	$\frac{1}{20}$th of a 24-hours old agar culture subcutaneously	$\frac{1}{5}$th of a 48-hours old agar culture subcutaneously	Bled	—	—	—
I. b.	,,	,,	,,	$\frac{1}{10}$th of a 48-hours old agar culture subcutaneously	Bled	—	—
I. c.	,,	,,	,,	,, ,,	,,	—	—
I. d.	,,	,,	,,	—	,,	—	Bled
I. e.	,,	,,	,,	$\frac{1}{5}$th of a 48-hours old agar culture subcutaneously	—	—	,,
I. f.	,,	,,	,,	,, ,,	$\frac{1}{5}$th of a 48-hours old agar culture subcutaneously	Bled	—
I. g.	,,	,,	,,	,,	,,	,,	—
I. h.	,,	,,	,,	,,	,,	—	Bled
I. k.	,,	,,	,,	,,	,,	—	,,
I. m.	,,	,,	,,	,,	,,	—	,,

TABLE II. *Showing the number of Colonies in the Typhoid Plates from Rabbits' sera.*

Age of serum	Drops of serum	Typhoid culture	N.1	N.2	N.3	N.4	N.5	I.a	I.b	I.c	I.d	I.e	I.f	I.g	I.h	I.k	I.m
1—2 hours	5	1 loopful	1,000	800	·	—	—	—	—	—	450	2	—	—	3,500	5,000	16,000
	10	„	400	250	—	—	—	300	600	300	300	1	—	—	1,600	2,500	8,000
	20	„	250	70	—	—	—	100	250	100	—	—	—	—	—	—	—
	30	„	—	—	—	—	—	—	150	20	—	—	—	—	—	—	—
4—6 hours	5	„	3,000	600	1,500	3,500	2,000	—	—	—	3	3	—	—	3,000	3,500	4,000
	10	„	2,000	120	700	1,500	800	20	600	100	1	1	—	—	1,400	1,800	1,200
	20	„	1,000	40	·	—	—	10	300	35	—	—	—	—	—	—	—
	30	„	—	—	—	—	—	—	150	10	—	—	—	—	—	—	—
24 hrs.	5	„	5,000	1,000	8,000	9,000	10,000	—	—	—	70	800	600	30,000	30,000	25,000	40,000
	10	„	2,500	200	3,000	4,000	4,500	300	2,000	1,500	20	400	30	25,000	10,000	6,000	6,000
	20	„	—	50	—	—	—	100	800	500	—	—	—	—	—	—	—
	30	„	—	—	—	—	—	—	300	200	—	—	—	—	—	—	—
2 days	5	„	16,000	5,000	20,000	unc.	unc.	20,000	30,000	15,000	10,000	7,000	2,500	unc.	unc.	50,000	unc.
	10	„	9,000	1,500	7,000	20,000	30,000	10,000	12,000	7,000	2,500	3,000	100	unc.	unc.	30,000	35,000
	20	„	—	300	—	—	—	—	—	—	—	—	—	—	—	—	—
	30	„	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3 „	5	„	unc.	8,000	50,000	∞	∞	·	unc.	—	10,000	55,000	12,000	∞	∞	unc.	∞
	10	„	unc.	5,000	30,000	unc.	unc.	unc.	unc.	50,000	2,500	40,000	8,000	∞	∞	unc.	unc.
	20	„	—	—	—	—	—	unc.	—	30,000	—	—	—	—	—	—	—
4 „	5	„	—	40,000	unc.	∞	∞	—	—	unc.	unc.	unc.	30,000	∞	∞	∞	∞
	10	„	∞	—	unc.	unc.	unc.	∞	∞	unc.	unc.	unc.	20,000	∞	∞	∞	∞
	20	„	∞	—	—	—	—	∞	∞	—	—	—	—	—	—	—	—
5 „	5	„	—	unc.	∞	—	—	—	—	—	∞	∞	unc.	∞	—	∞	∞
	10	„	—	—	∞	—	—	∞	∞	∞	∞	∞	unc.	∞	—	∞	∞
	20	„	—	—	—	—	—	∞	∞	∞	—	—	—	—	—	—	—
6 „	5	„	—	—	∞	—	—	—	—	—	∞	—	∞	—	—	—	—
	10	„	—	∞	∞	—	—	—	—	∞	∞	—	∞	—	—	—	—
	20	„	—	—	—	—	—	—	—	∞	—	—	—	—	—	—	—
∞ „	5	„	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	10	„	—	∞	—	—	—	—	—	∞	—	—	—	—	—	—	—
	20	„	—	—	—	—	—	—	—	∞	—	—	—	—	—	—	—

unc. = uncountable; ∞ = infinite

observations, so that the succeeding observations in each series are strictly comparable; and all the observations given in Table II. were carried out by means of the same pipette and are therefore comparable throughout. These pipettes were blown for the purpose and were drawn out in such a way as only to allow the escape of fluid in drops even when the finger was entirely removed from the upper end. The size of the drops from any one pipette was therefore approximately constant. In order to obtain an estimate of the amounts of serum that were being used, a number of the pipettes were measured as regarded the volume of the drops which they allowed to escape. And it was found that the volume of 300 drops varied with different pipettes between 8·6 and 9·4 cubic centimetres, the average being about 9 cubic centimetres. Twenty drops were therefore equivalent to about 0·6 cubic centimetres of the fluid employed.

The tubes of serum which had been measured in the manner just described were next inoculated each with one loopful of a culture of typhoid bacilli grown for about 24 hours in 10 cubic centimetres of bouillon. The tubes were incubated at 37° C. for a period of three hours[1], to each was then added 10 cubic centimetres of melted gelatin culture-medium, and the contents thoroughly mixed and plated. Colonies were counted in the plates after three days' growth. In every case a control plate was made with uninoculated serum to test its sterility, and another with one loopful of the typhoid culture inoculated and incubated for three hours in 0·5 cubic centimetres of ordinary bouillon for comparison with the plates from inoculated serum.

The colonies were enumerated by means of a Wolffhügel counting apparatus. Where they were very numerous they were estimated approximately. Up to 100 or more colonies per square cm. could be counted with a lens with moderate accuracy. Above this number I have used the term "uncountable," where the colonies were obviously less numerous than those in the control without serum, and the sign ∞ (infinite) where no such difference was appreciable.

The results of the first series of observations made on fresh rabbits' serum are given in Table II.

In the foregoing Table II., N. 1 to N. 5 are the sera of the five normal rabbits, and I. *a.* to I. *m.* are the sera of the rabbits which had been inoculated with the *Bacillus typhosus* as shown in Table I.

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[1] The time three hours was chosen for convenience because it was found that at this period the number of colonies which grew in the control plates from ordinary bouillon had become innumerable, and therefore formed a constant standard for comparison.

1. The Table II. shows quantitatively the progressive disappearance of the complement (addiment) from separated serum. The fact that complement thus suffers a rapid disappearance is of course an old observation, but I am unable to discover that any attempt has previously been made to study the time relations of the process.

2. The Table also shows that while there is some difference between different sera in the rapidity with which the complement disappears, no evident differences appear between the sera of normal and of immunised animals in this respect.

3. The bacteriolytic power *in vitro* of the sera of the immunised animals was markedly greater than that of the normal sera, except in the case of I. *h.*, I. *k.*, and I. *m.* These were the sera of the animals already referred to which were killed while in a condition of pyrexia and impaired nutrition following the inoculations. In them the bacteriolytic action of the serum was very definitely less than that of normal uninoculated animals.

4. I. *f.* and I. *g.* were the sera of two rabbits of about the same age and weight, which had been similarly treated (see Table I.) and which were bled at the same time. The serum I. *f.* was kept continuously in an ice-chest; I. *g.* and all the other sera being kept at the ordinary room-temperature of the laboratory (about 14° C.) but in the dark. The serum I. *f.* was not separated from the clot at the end of 24 hours as were the other sera but was left in contact with its clot throughout, only so much as was required for observation being removed each day. It shows a marked retardation of the disappearance of the bacteriolytic power.

On comparing the bacteriolytic power of serum taken from the clot from one to two hours after the blood had been collected with that of serum of the same animal removed from two to four hours later (*i.e.* four to six hours old) from the same tube a very remarkable fact was to be observed in many of the sera, namely, that *the bacteriolytic power was greater at the second observation than at the first.* That is to say that the amount of complement in the serum had apparently undergone an increase during the early hours of the separation of the serum from the clot. In order to verify this observation I have proceeded to examine a number of normal sera such as could be readily obtained in quantity, using the same method as before and estimating their bacteriolytic action at each succeeding hour. Sheep's serum was the most easily available, and several specimens of this were obtained on different occasions and tested hourly for the first six hours from the

time of slaughtering. This with the subsequent three hours' incubation followed by plating requires from nine to ten hours continuous work, and therefore as the sheep were only killed at the slaughter-house between 3 and 4 o'clock in the afternoon it was not found convenient to extend the observations further the same night.

As already stated the method followed was the same as in the case of the rabbits' sera above, but here the typhoid culture was used in each

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TABLE III.

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Serum and whipped blood of sheep, showing the number of colonies which grew in each plate.

		Streptococcus Plates		Bacillus Typhosus Plates				
Age of serum or whipped blood	Quantity of serum or whipped blood	Strepto-coccus culture about 8 hours old	Colonies from serum tubes of sheep 1	Typhoid culture about 8 hours old	Colonies from serum tubes of sheep 1	Colonies from serum tubes of sheep 2	Colonies from whipped blood tubes of sheep 3	Colonies from whipped blood tubes of sheep 4
1 hour	20 drops	1 loopful	100,000	1 loopful	65	35	8	10
2 hours	,,	,,	44,100	,,	23	20	11	10
3 ,,	,,	,,	37,800	,,	19	12	9	13
4 ,,	,,	,,	33,500	,,	16	23	15	17
5 ,,	,,	,,	31,900	,,	13	28	22	21
6 ,,	,,	,,	36,700	,,	17	33	31	33
18 ,,	,,	—	—	,,	—	62	—	—
20 ,,	,,	—	—	,,	51	—	—	—
4 hours taken from clot at 1 hour	,,	—	—	,,	—	34	—	—
5 hours taken from clot at 1 hour	,,	—	—	,,	—	42	—	—
5 hours taken from clot at 2 hours	,,	—	—	,,	35	—	—	—
5 hours taken from clot at 3 hours	,,	—	—	,,	26	—	—	—
18 hours taken from clot at 5 hours	,,	—	—	,,	—	495	—	—

Control plates of uninoculated serum all sterile.

case, one planted in broth early on the morning of the experiment and
incubated until required the same evening. On some occasions the
sera were tested also against cultures of *Streptococcus*. The serum was
left in contact with the clot throughout, a sample being removed each
hour for examination. In some cases also some of the serum was
removed from the clot after one hour or several hours and left to
stand in a test-tube for comparison at a later period with a sample of
serum freshly removed from the flask containing clot and serum. On
two occasions instead of serum whipped blood was obtained and sub-
mitted to a similar examination. The most satisfactory method of
obtaining sterile whipped blood for this purpose was found to be by
receiving the blood into an Erlenmeyer flask into which a small spiral
coil of iron wire had been introduced before sterilisation, the flask
being kept in gentle agitation until the deposition of fibrin was
completed.

In Tables III. and IV. the results obtained with two sheep sera and
with whipped blood from two other sheep are recorded, as well as those
with the serum and whipped blood of the same rabbit.

TABLE IV.

*Serum and whipped blood of Rabbit A, showing the number of colonies
which grew in each plate.*

Age of serum or whipped blood	Quantity of serum or whipped blood	Typhoid culture about 16 hours old	Colonies from serum tubes	Colonies from whipped blood tubes
1 hour	20 drops	1 loopful	600	150
2 hours	,,	,,	480	170
3 ,,	,,	,,	350	210
4 ,,	,,	,,	240	280
5 ,,	,,	,,	270	340
6 ,,	,,	,,	330	430
6 hours taken from clot at 1 hour	,,	,,	810	—
6 hours	,,	0	0	0

These Tables (III. and IV.) show incidentally the very marked
difference between the bacteriolytic power of normal sheep's blood
for typhoid bacilli and that for *Streptococci*. They also show that
while the bacteriolytic power of whipped blood is originally somewhat
greater than the maximum attained by serum (cp. Table IV.) yet it
diminishes progressively with each succeeding hour, while on the other

hand *the bacteriolytic power of serum which is left in contact with the clot progressively increases during the earlier hours after the blood is shed, and only subsequently begins to undergo a diminution.* And that this increase is associated with the presence of the clot follows from a comparison of the observations on specimens of serum taken from the flask containing the clot at a given hour but only tested at a later period after some hours' standing with those on specimens freshly removed from the clot-containing vessel and submitted to examination at the same later period as the former. Thus to take the two specimens of serum in Table IV. which were examined for bacteriolytic power at the sixth hour, the first, which was freshly taken from the clot for this examination, only showed 330 colonies on plating after the usual procedure, while the second, which had been removed at the first hour—at a time when the bacteriolytic power was such that some 600 colonies were formed upon the plate from serum examined at that age—and had been left to stand in a test-tube for the next five hours, allowed the growth of 810 colonies after similar treatment.

Nuttall[1] in 1888 was the first to work upon the bacteriolytic action of fresh serum. He showed that lysogenesis depends upon some property of the serum which is destroyed by the application of a temperature of from 52° to 55° C. Following the appearance of Nuttall's paper came a numerous series of experiments by Buchner[2], Lubarsch and others, in none of which, however, does any exact consideration of the age of the serum used appear to have been taken into account. Behring and Nissen[3] have also published numerous results of their experiments on the bacteriolytic action of fresh sera. These sera were usually employed of the age of two, four, or six hours from the time of bleeding, but no comparison was made of the action of one and the same serum at succeeding periods. Von Fodor[4] showed that in defibrinated dog's blood the bacteriolytic power for anthrax steadily decreases from the time the blood is shed. He concluded that this progressive loss is due to changes leading to the production of acid in the shed blood; and showed that the bacteriolytic power of blood may be increased by previous administration of various alkaline substances, which he believed delayed this gradual process of acidification. The salts which von Fodor

[1] *Zeitschrift für Hygiene*, 1888, IV. p. 353.
[2] *Centralblatt für Bakteriologie*, 1899, V. and VI. *Fortschritte der Medicin*, 1892, Vol. X. pp. 9, 10.
[3] *Zeitschrift für Hygiene*, 1890, VIII.
[4] *Centralblatt für Bakteriologie*, 1890, VII. p. 753.

administered were however such as have also the effect of producing a considerable leucocytosis.

Considering the results of the experiments which I have here recorded, it seems quite clear that while in a whipped blood the whole available bacteriolysin of the blood is of course present from the outset, and undergoes a steady diminution or degeneration from the first; that of the serum while progressively deteriorating also, is in the earlier hours continually receiving fresh additions from the clot. Accordingly the evidence supports most strikingly the view that *the bacteriolytic "ferment" is a leucocytic product*, and is yielded to the serum by the gradual disintegration of the leucocytes during and subsequently to coagulation of the blood. Moreover this accords entirely with the experiments which have been published by Bordet and Gengou[1] showing that *plasma* rapidly separated without appreciable destruction of leucocytes has practically no bacteriolytic action. It is completely in antagonism with the view which was proposed by Ehrlich that the bacteriolytic "ferment" is a substance normally present in the plasma of the living animal.

During the progress of the first series of observations (*vide* Table I.) I was anxious to determine how far, if at all, the amount of immune body produced by the inoculated rabbits exceeded their available complement (addiment). Accordingly I took a series of tubes containing equal amounts of fresh rabbits' serum of six hours' age and added to them different quantities of rabbits' immune serum which had been heated for an hour at 55° C. The tubes were then inoculated, incubated and plated in the usual way. Some of the plates gave the unlooked-for result which is shown in Table V. below.

TABLE V.

Showing the effect on bacteriolytic power of the addition to fresh serum of increasing quantities of inactivated immune serum.

Fresh rabbits' serum	Serum I. g. heated for 1 hour at 55° C.	Typhoid culture	Colonies in plates
10 drops	2 drops	1 loopful	360
,,	5 ,,	,,	1,920
,,	10 ,,	,,	48,000
,,	0	0	0

[1] *Annales de l'Institut Pasteur*, 1901.

Here it appeared that the more immune serum had been added to the fresh normal serum the greater was the number of colonies which grew in the plates; in other words, that an excess of immune body had the effect of lessening the bacteriolytic action exercised.

This fact I found had been already recorded by Neisser and Wechsberg[1], who (1901) state that both in the living animal and in the test-tube an excess of immune serum is prejudicial to bacteriolytic action. I have since made a number of observations on this question

TABLE VI.

Showing the effect upon bacteriolysis of the addition to fresh serum of increasing quantities of inactive immune serum.

Fresh rabbits' serum	Inactive rabbits' antityphoid serum. Drops	Typhoid culture	Colonies in plates
10 drops	0	0	0
,,	2	1 loopful	400
,,	5	,,	1,800
,,	10	,,	45,000
,,	20	,,	unc.
,,	40	,,	∞
,,	80	,,	∞
,,	0	,,	830
5 drops	0	,,	1,960

unc. = uncountable :   ∞ = infinite.

TABLE VII.

Showing the effect upon bacteriolysis of the addition to fresh serum of a rabbit of increasing quantities of inactive immune serum of a horse.

Fresh rabbits' serum	Inactive horse's antityphoid serum. Drops	Typhoid culture	Colonies in plates
10 drops	0	0	0
,,	0	1 loopful	1,080
,,	1	,,	420
,,	5	,,	1,320
,,	25	,,	50,000
,,	50	,,	unc.
,,	100	,,	∞

unc. = uncountable :   ∞ = infinite.

[1] *Münchener medicinische Wochenschrift*, 1901, Vol. XLVIII. p. 679.

with the fresh sera of the rabbit and the sheep and old[1] immune sera of the rabbit and the horse. The results, which serve to confirm the discovery of Neisser and Wechsberg, are given in Tables VI. VII. and VIII.

TABLE VIII.

Showing the effect upon bacteriolysis of the addition to the fresh sera of sheep 1 and 2 of increasing quantities of inactive immune serum of the rabbit and of the horse.

	Fresh serum 4 hours old. Drops	Inactive rabbit's antityphoid serum. Drops	Typhoid culture	Colonies in plates	Fresh serum 4 hours old. Drops	Inactive horse's antityphoid serum. Drops	Typhoid culture	Colonies in plates
Fresh serum of sheep 1	20	0	0	0	—	—	—	—
	0	20	0	0	0	20	0	0
	20	0	1 loopful	15	20	0	1 loopful	17
	0	20	,,	∞	0	20	,,	∞
	20	1	,,	12	20	1	,,	0
	20	5	,,	2	20	5	,,	10
	20	10	,,	20	20	10	,,	300
	20	20	,,	420	20	20	,,	3,600
	20	40	,,	5,400	20	40	,,	27,000
	20	60	,,	32,400	20	60	,,	70,000
	20	80	,,	54,000	20	80	,,	unc.
	20	100	,,	unc.	20	100	,,	∞
	20	140	,,	∞	20	150	,,	∞
Fresh serum of sheep 2	20	0	0	0	—	—	—	—
	0	20	0	0	—	—	—	—
	20	0	1 loopful	30	20	0	1 loopful	28
	0	20	,,	∞	0	20	,,	∞
	20	1	,,	5	20	1	,,	6
	20	5	,,	2	20	5	,,	50
	20	10	,,	17	20	10	,,	175
	20	20	,,	540	20	20	,,	6,000
	20	40	,,	6,000	20	40	,,	36,000
	20	60	,,	42,000	20	60	,,	unc.
	20	80	,,	unc.	20	80	,,	∞
	20	100	,,	∞	20	100	,,	∞

unc. = uncountable: ∞ = infinite.

Observations were also made with antistreptococcic immune sera, these were the sera of two horses immunised by Professor Tavel in Berne, and are denominated as S.1. and S.2. I had tested them when fresh as regards bacteriolytic power for *Streptococci* with the results

[1] 2 to 4 months old for the rabbits' antityphoid sera; and about 1 year old for the horse's antityphoid serum.

Table IX.

Showing the bacteriolytic power of the horses' antistreptococcic sera S.1. *and* S.2. *when fresh compared with that of the normal rabbits' serum* N.4.

Age of serum	Drops of serum	Streptococcus culture	Serum N.4. Colonies in plates	Serum S.1. Colonies in plates	Serum S.2. colonies in plates
1 hour	5	1 loopful	∞	——	50,000
	10	,,	∞	—	16,000
3 hours	5	,,	∞	1,800	—
	10	,,	∞	900	—
4 ,,	5	,,	∞	—	10,000
	10	,,	∞	—	6,000
6 ,,	5	,,	∞	40,000	—
	10	,,	∞	16,000	—
24 ,,	5	,,	∞	unc.	unc.
	10	,,	∞	50,000	40,000
2 days	5	,,	∞	∞	∞
	10	,,	∞	unc.	unc.
3 ,,	5	,,	∞	∞	∞
	10	,,	∞	∞	∞

unc. = uncountable : ∞ = infinite.

exhibited in Table IX. They were already four months old when used for the observations given in Tables X. and XI.

Table X.

Showing the effect on bacteriolysis of the addition to fresh rabbits' serum of increasing quantities of the now inactive immune serum S.1. *of a horse.*

Rabbits' serum, 4 hours old. Drops	Inactive horse's antistreptococcus serum S.1. Drops	Streptococcus culture	Colonies in plates
15	0	1 loopful	32,400
15	1	,,	15,600
15	5	,,	6,500
15	25	,,	13,200
15	50	,,	54,000
15	100	,,	∞

∞ = infinite.

TABLE XI.

Showing the effect upon bacteriolysis of the addition to the fresh sera of sheep 1 and 2 of increasing quantities of the now inactive horses' immune sera S.1. and S.2. respectively.

Fresh serum of sheep 1, 4 hours old. Drops	Inactive horses' antistreptococcus serum S.1. Drops	Strepto-coccus culture	Colonies in plates	Fresh serum of sheep 2, 4 hours old. Drops	Inactive horses' antistreptococcus serum S.2. Drops	Strepto-coccus culture	Colonies in plates
20	0	1 loopful	43,200	20	0	1 loopful	unc.
0	20	,,	∞	0	20	,,	∞
20	1	,,	13,500	20	1	,,	unc.
20	5	,,	8,100	20	5	,,	32,400
20	10	,,	16,300	20	10	,,	14,000
20	20	,,	24,300	20	20	,,	34,000
20	40	,,	35,100	20	40	,,	100,000
20	60	,,	81,000	20	60	,,	unc.
20	80	,,	unc.	20	80	,,	∞
20	100	,,	∞	20	100	,,	∞
20	150	,,	∞	20	150	,,	∞
20	200	,,	∞	20	200	,,	∞

unc. = uncountable : ∞ = infinite.

The remarkable diminution of bacteriolytic power occasioned by the presence of an excess of immune serum is shown in each of the Tables VI. VII. VIII. X. and XI. where the effect of the addition of increasing quantities of immune serum is seen to be at first a marked increase, but later when the amounts become excessive a progressive loss of the bacteriolytic action of the normal serum. An explanation of this phenomenon can be suggested, as was pointed out by Neisser, which is compatible with the views of Ehrlich on the mechanism of the bacteriolytic process, while no such explanation can be found consonant with the various other theories of the nature of bacteriolysis which are at present held by different writers. This explanation may be briefly stated thus.

Complement and immune body possess a certain mutual affinity which may conceivably be increased or lessened by a variety of in-fluences. The attachment of the immune body to a bacterium is such an influence. If then bacteria be brought into a fluid containing their specific immune body and also complement, the former as we know becomes attached to the bacteria, and its affinity for the complement may

thus be modified. The case presents three possible alternatives as follows. The attachment of the immune body to bacteria may (*a*) increase its affinity for the complement; it may (*b*) cause no alteration at all in this affinity; or it may (*c*) diminish the affinity for complement.

If the first of these alternatives held good, no serious consequence could attend the presence of excess of immune body. For that part of it which had attached itself to the bacteria, would, by its heightened affinity for complement, be enabled to secure that substance by its complement-haptophore and the destruction of the bacteria would follow. But if the second, and much more if the third alternative holds good the free excess of immune body present will have either (1) as great, or (2) an even greater affinity for complement than that which is already attached to the bacteria. It follows, therefore, that if the third alternative correctly represents the situation, or if the second does so and a great excess of immune body be at hand, practically the whole available complement will be secured by the free immune body, and none, or very little, by the immune body already fixed in the bacteria. Bacteriolysis will therefore fail from lack of complement. This is a possible explanation of the situation in the experiments recorded. But I am not convinced that it is altogether satisfactory. Probably other factors still require investigation. The facts observed, however, appear to be unquestionable.

These observations help to explain the experience of Pfeiffer and others who have found that highly immunised animals may at times succumb to moderate multiples of the M. L. D.[1] of the bacterium against which they have been immunised; and certainly suggest the necessity of caution in the administration of large quantities of anti-microbic sera for therapeutic purposes in the human subject. And it would appear to be at least an equally important object, failing the eventual discovery of some means of introducing an additional supply of complement from external sources, to *secure the presence of adequate leucocytosis* in all conditions of infective origin, and more especially *whenever the injection of anti-microbic serum is contemplated.*

I draw the following conclusions from the observations which have been here recorded.

1. The amount of complement present in a given serum varies continuously from hour to hour after the blood is shed. It undergoes a steady increase during the first few hours if the serum be left in

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[1] M. L. D. = Minimal lethal dose.

contact with the clot, and only subsequently begins to show progressive diminution. Serum removed from the clot-containing vessel and whipped blood, on the other hand, show no such increase of their complement, which undergoes a steady diminution from the first.

2. Complement (addiment) is a leucocytic product only appearing in blood-plasma or serum as the result of a disintegration of leucocytes.

3. Observations on the bacteriolytic action of a serum are only comparable *if performed with the same serum and at the same time.*

4. The exhibition of excess of immune-serum may be as harmful in the course of an infection as its entire omission ; and great excess of immune-serum might perhaps directly bring about a fatal issue by absorbing all the complement and thus arresting normal protective processes.

THE BEARING OF OUTBREAKS OF FOOD POISONING UPON THE ETIOLOGY OF EPIDEMIC DIARRHOEA.

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Introduction.

It is almost impossible to avoid some confusion when one has to deal with diseases which it is difficult to define clearly either by means of *symptoms* and *lesions,* or by means of some *causal agent* capable of demonstration in the majority of cases.

Diarrhoea is a symptom produced by a number of causes, and each one of these causes according to its *intensity* of action, or to the *state of the patient* may produce several types of diarrhoea.

Moreover, there is evidence to show that the agent which initiates an attack of diarrhoea may in the course of the illness caused by its action be superseded by other agents (*e.g.* certain bacteria generally present in the intestine), by which the course of the disease may be so completely altered as to make it difficult to recognise the relations between cause and effect.

In bringing forward certain facts which in my opinion establish clearly certain relations between food poisoning and epidemic diarrhoea, I must labour under a double difficulty, for neither food poisoning nor epidemic diarrhoea is a well-defined pathological entity.

Definition of Epidemic Diarrhoea.

By epidemic diarrhoea is generally meant *an infectious disease affecting a number of persons at the same time, more specially during the hot seasons,* and *the most constant symptom of which is diarrhoea.* One of its special features is its tendency to occur in certain poor populous districts, where it causes great mortality among children.

From descriptions I have been able to find in standard books of reference it appears to me that such terms as *English cholera, Cholera nostras, Cholera infantum, Choleraic* or *Choleriform diarrhoea, Septic diarrhoea, Summer diarrhoea, Infantile diarrhoea,* etc. must, generally speaking, be taken as synonyms of epidemic diarrhoea. To the morbid anatomist epidemic diarrhoea is an acute *gastro-enteritis.*

Chief Symptoms.

Epidemic diarrhoea is characterised by a certain number of symptoms, which have been summed up by Dr Ballard as follows:

The leading phenomena of the disease are diarrhoea, vomiting, convulsive phenomena; a bodily temperature at certain periods above, at other periods below, what is normal; reduction in quantity, or actual suppression of urine, embarrassed breathing, indications of pulmonary hyperaemia or inflammation, pallor of surface of the body, loss of bulk and flesh, and exhaustion with its well-known clinical features. Occasionally there is jaundice; now and then a fugitive rash has been observed on the body.

These symptoms agree with those given by various clinical authorities as characteristic of English cholera and infantile diarrhoea, but clinicians include intense thirst, pain and griping, cramps, bilious vomiting and diarrhoea among the important symptoms. Suppression of urine, convulsions and cramps are not among the characteristic features of the ordinary milder attacks of the disease.

Severe cases of the same disease may present features almost identical with those of Asiatic cholera, poisoning by certain organic and inorganic poisons, or even perforation of the stomach or bowel.

The difficulty of diagnosis of isolated cases is very considerable, and the nature of the disease is generally indicated by its epidemic and seasonal characters more than by any semeiotic feature.

Morbid Anatomy.

In fatal cases the lesions observed are not sufficiently characteristic to remove all doubts. Among post-mortem changes I may mention, absence of food in the stomach and small intestine, hyperaemic, swollen, sometimes ulcerated mucous membrane of the same parts. Accumulation of mucus, frequently bile stained, upon these mucous membranes. Degenerative changes in the liver and kidney. Hyperaemia of the

lungs, sometimes pneumonia. The spleen is variable in size and frequently small. There is nothing pathognomonic in any of these lesions.

Bacteriology of Epidemic Diarrhoea.

Exact notions regarding the bacteriology of forms of enteritis other than those connected with cholera, typhoid fever and anthrax, were very few before the year 1885, when the classical researches of Escherich upon the *Bacillus coli communis* became known. The researches of that observer showed that although the *Bacillus coli communis* is a constant inhabitant of the intestine, it is capable under certain circumstances of acquiring pathogenic properties. Hueppe in 1887 stated that it was capable of producing cholera nostras, a view which has also been supported at a later date by Gilbert and Girode and others. Lesage, Macé and Simon, Cumston, etc. have found the *Bacillus coli communis* in a large proportion of the cases of infantile diarrhoea which they have investigated, and believe that organism to be the cause or the most important cause of summer diarrhoea in children.

Other observers however have attributed to other organisms an important share in the production of epidemic diarrhoea.

As far back as 1884, Finckler and Prior found in cases of cholera nostras the *Spirillum* which has received their names.

Baginski, in Germany, Booker, in America, found in the stools and organs of children affected with infantile diarrhoea a number of micro-organisms, among which may be mentioned the *Bacillus lactis aerogenes*, the *Bacillus coli communis*, the *Proteus vulgaris*, Staphylococci, Streptococci, etc.

Booker does not believe that the summer diarrhoea of infants is due to any single organism, but nevertheless he regards with suspicion the *Proteus vulgaris*, and the *Streptococcus enteritidis*. Holst evidently attaches importance to the streptococci, for he has recorded in connection with several epidemics of summer diarrhoea, the presence of the *Streptococcus longus* in cows' milk which had been used by the patients. A streptococcus has also been found in connection with gastro-enteritis in adults.

Damaschino, Clado and Lesage have described a bacillus producing a green pigment, as the cause of a green diarrhoea of infants.

Klein attributes to the *Bacillus enteritidis sporogenes* which he has found in the intestines of children suffering from diarrhoea, an important share in the production of the disease.

This short and incomplete review is sufficient to show that the etiology of epidemic diarrhoea is as yet by no means clear.

It will be noticed, however, that the bacteria which have been suspected on good grounds of being the cause of outbreaks of summer diarrhoea belong chiefly to types of bacteria which are inhabitants of the alimentary canal. The virulence of several of these microbes has been shown to be capable of considerable variation.

The bacilli of the colon group appear to be most intimately connected with the epidemic diarrhoea both of adults and of children.

These bacilli resemble each other so closely in some respects that one is tempted to regard them as varieties of the same stock. Yet if one submits colon bacilli obtained from the intestinal contents of a number of cases of diarrhoea to the series of tests which are usually employed for diagnostic purposes, it is found that one or more of the reactions which are associated with the *Bacillus coli communis* of Escherich are frequently absent. The acidity which is generally produced very early in various media may be permanent, or replaced more or less rapidly by an alkaline reaction; milk is sometimes coagulated rapidly, sometimes very slowly or not at all, similar variations are observed with regard to the indol reaction, the fermentation of various sugars, the appearances of the growth on potato, and even the serum reaction. The serum reaction presents with regard to each of the many races of the *Bacillus coli* such a degree of specificity that it is deprived of much of its practical value for the purpose of a clinical diagnosis. In the investigation of limited outbreaks due to a definite source of infection this reaction may, however, be very useful for the purpose of determining whether a bacillus isolated from the implicated material or from some of the cases is the actual cause of the outbreak. It must however be remembered that the blood of patients suffering from this kind of infection gives a definite reaction only for a very limited period of time, and in very severe cases the serum reaction is generally very indistinct or absent.

I cannot in the time at my disposal discuss this aspect of the question at greater length[1].

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[1] A recent paper by Dr M. H. Gordon gives a fairly complete account of our present knowledge of the Bacteriology of Epidemic Diarrhoea. *Practitioner*, August, 1902.

Epidemiological features of Epidemic Diarrhoea.

The epidemiological features of summer diarrhoea so well brought out by Ballard's investigation would therefore appear for the present to constitute the most satisfactory proofs of the specific character of summer diarrhoea.   I will refer to them at a later stage.

Food Poisoning.

The resemblance between many outbreaks of food poisoning and epidemic diarrhoea is very great; this resemblance would be even clearer if certain special forms of food infections such as botulism were entirely separated from the general mass of cases.

Van Ermengem describes under the name of *botulism* a state brought about by the ingestion of various articles of food such as ham, tinned or preserved foods, oysters, mussels, etc., which is characterised by comparatively slow onset (12 to 24 hours after infection), secretory troubles, paralysis of certain muscles, dysphagia, constipation, retention of urine, absence of fever, etc.   Van Ermengem has found that these symptoms were produced by a bacillus to which he has given the name of *Bacillus botulinus.*

Botulism differs considerably from the more *common form of food poisoning* with which we are specially acquainted in this country.

This last form of food poisoning is however very imperfectly understood, as may be seen by consulting our chief books of reference. Observers are generally of the opinion that in the large majority of cases food poisoning results from the ingestion of the flesh of animals suffering from certain forms of septicaemia, enteritis, or pneumoenteritis, whose flesh owes its noxious properties to various bacilli resembling more or less closely the *Bacillus enteritidis* of Gaertner. There is good evidence to show that this kind of infection occurs at times, but I will show that the mode of infection is usually different, and the result of a more general kind of contamination.

Works on Hygiene and Public Health attribute the outbreaks of diarrhoea which usually attract attention, mostly in connection with hospitals and other public institutions, to the consumption of *water* containing an excess of mineral waters, or contaminated with sewage; to *milk* which has been exposed to effluvia in ill-ventilated places, or which has undergone fermentation; to *tinned meats, pork-pies, ham, game, fish, cheese.*   In the latter cases the symptoms are usually

supposed to be the results of the action of some chemical poison (Ptomaines, Tyrotoxicon, etc.) produced during putrefaction, so that the disease is generally described under the name of *ptomaine poisoning,* a name which I consider to be misleading in the great majority of cases.

Outbreaks of diarrhoea due to the consumption of food are not clinically distinguishable as a group from summer diarrhoea or cholera nostras. Thus Taylor tells us that *acute gastro-enteritis (English cholera*), which occurs occasionally during the summer months, is set up by unsuitable ingesta such as sausages, meat, pies, shell-fish, or food in a state of decomposition. He also says that there is reason to believe that some of these cases are due to *ptomaine poisoning.* The similarity of the causes of food poisoning and summer diarrhoea is clearly indicated by a great number of clinicians.

Thus although we have grown accustomed to designate by different names certain types of diarrhoeal diseases, it is obvious that these various names do not indicate a reasonable belief in the existence of so many diseases, but relate rather to the *mode of occurrence of a single disease, or of a small group of closely allied diseases.* General evidence seems to show that the most common forms of food poisoning give rise to symptoms resembling closely those of epidemic diarrhoea. Outbreaks of food poisoning may occur at all times of the year, but they are generally most frequent at the time when epidemic diarrhoea is most prevalent. That they affect adults as well as infants appears to be due chiefly to the kinds of food which produce them, but certain foods, such as milk, which are partaken of both by infants and adults, give rise in both to attacks of diarrhoea similar to typical attacks of epidemic diarrhoea of unknown origin.

Now I consider that the chief difficulty which workers have experienced in searching for the actual cause of epidemic diarrhoea, is due to the fact that they have looked for it in the alvine discharges, or in the organs of patients who have died from the disease.

If, as is reasonable to believe, the bacteria causing epidemic diarrhoea are intestinal bacteria, it is obvious that a great difficulty must be experienced in determining which of these bacteria is responsible for the disease. Many of these bacteria are virulent when introduced into the tissues, many of them increase in virulence in the course of an attack of diarrhoea, and several of them have a tendency to escape from the intestine and penetrate into the various tissues of the body immediately after death or even during the last hours of life at the end of an exhausting illness.

On the supposition that epidemic diarrhoea is generally the result of a more *widely disseminated, and less massive form of bacterial infection of food* than is the case with regard to the more definite outbreaks of food poisoning, we should be able to utilise the occurrence of certain outbreaks of food poisoning for the purpose of turning the difficulties which we have generally to contend with in the investigation of epidemic diarrhoea.

It is with that object that I have for the past eight years investigated the action of bacteria which had given rise to outbreaks of food poisoning, and have also studied certain pathogenic properties of the cows' milk which is supplied to our towns, and which is responsible for much of the infantile mortality due to epidemic diarrhoea. During these eight years I have had opportunities to study several outbreaks of infection due to milk, cheese, pork-pies, tinned salmon and other foods. I have also been able to study the pathogenic action of over 2000 samples of milk upon guinea-pigs. By comparing the properties of bacilli obtained from noxious articles of food, and of those obtained from animals which had suffered from the effects of inoculation with various samples of milk, I have been able to satisfy myself that these bacilli, with few exceptions, belonged to the colon group of bacilli.

To show the nature of the evidence obtained I will now give a short account of three of these investigations. A more complete analysis of the statistical data I have accumulated cannot yet be attempted.

Manchester Outbreak of Diarrhoea due to the Consumption of Milk, November 1894.

In the early part of the month of November 1894 there occurred in Manchester an extensive outbreak of diarrhoea attributable to the consumption of milk. This outbreak was investigated by Dr Niven, from whose report I take the following particulars. (Report on the Health of Greater Manchester, 1894, pp. 102 *et seq.*)

On Nov. 7 Dr H. Ashby sent a note to Dr Niven in reference to a number of cases of illness which had occurred in Victoria Park, Manchester. The symptoms were those usually associated with English cholera. Those attacked were all supplied by one milk dealer, and Dr Ashby was of opinion that circumstances pointed to milk being the cause of the outbreak. Dr Niven ascertained that the attacks in the great majority of instances had occurred on the night of November 5, and in the early part of November 6. As a rule the persons attacked

had partaken of unboiled milk. One lady however was of opinion that all the milk brought into her house had been boiled. About 8 or 9 hours elapsed between the taking of the incriminated milk and the occurrence of symptoms of illness.

Altogether 160 cases were reported, occurring in 47 families; none of these proved fatal, but in several instances the symptoms were severe.

I need not enter here into a consideration of the steps taken by Dr Niven to establish the relationship between the outbreak and the milk supplied from one farm. The evidence so collected established clearly the relation.

On visiting the farm, Dr Niven ascertained that close to the farm-house there was a tip of *midden-privy refuse*, which was estimated to contain 40,000 tons of material of that kind. The farm was bordered by two streams which met below. The one coming from the tip was very foul, the other comparatively clear, but also contaminated with sewage and with matter from a *tripe-boiling place*. The water used to wash the pails was tepid: its temperature taken by Dr Martin on one occasion was 92° F. The water used in cleansing the milk-pails was kept in a foul cistern. The cows drank from a pool in the yard which received drainage from the cowshed midden. The storage of milk over-night was such as to expose it to warmth and contamination from the cowsheds.

Whilst this investigation was proceeding I was making a bacteriological examination of the only sample of suspected milk which could be obtained on the 8th of November. I will refer again to this, but it is convenient to state here that without any knowledge of what Dr Niven was finding, I reported to him that the *Bacillus coli communis* which was abundant in the milk, indicated sewage or faecal pollution, and that it was probable that some contaminated water had found its way into some of the vessels used for collecting or distributing the milk.

Meanwhile Dr Niven on continuing his investigation discovered a fact which had originally been concealed from him, viz., that on Nov. 8 a cow affected with inflamed udder (garget) had been removed from the farm and slaughtered on Nov. 10.

Several cases of bacterial infection due to the consumption of milk had been recorded in Germany, and in those cases the infection of the milk had been attributed to some disease of the cow.

On this basis Dr Niven concluded that the outbreak in Manchester was probably due to the disease of the cow which had been removed

from the farm.   It is probable that the farmer on hearing complaints from his customers on the 6th and 7th of November got alarmed, and knowing that one of his cows was affected with garget, disposed of it at once to remove evidence of culpable neglect on his part.

Thus it came to pass that the Victoria Park outbreak was attributed to some affection of the udder of one cow, a conclusion which had the support of opinions expressed with regard to some previous outbreaks of the same kind.

Results of the Bacteriological Examination of the Milk.

The examination of milk led me however to a different conclusion.

The only sample of the milk which had produced illness was 3 days old when it reached me, it was firmly clotted, strongly acid, and had an unpleasant, sour smell.   Six adults or adolescents and an infant who had partaken of this milk had been taken ill (with violent diarrhoea, sickness and pain) after a period of incubation lasting about 12 hours.

I gave large doses of this milk to two guinea-pigs and two young rats without producing any distinct illness in those animals.

On microscopical examination *very few cells* were found, *no pus*, the fat globules were partly confluent.   A very large number of bacteria were found, the most abundant being short, very motile bacilli resembling the *Bacillus coli*, large discrete *Cocci*, and a *Streptococcus* with large segments[1].   Anaerobic and aerobic cultures in milk and bouillon yielded a very abundant growth of several organisms.   I was specially struck with the abundance of bacilli which belonged to the *Bacillus coli* group.   Of these there were 3 varieties, one (A) having all the cultural characters of the typical *Bacillus coli communis*; another (B) which gave all the usual chemical cultural reactions of the *Bacillus coli communis*, but which produced on gelatine, thick colonies with rough mammillated surface; a third one (C) had most of the characters of the *Bacillus enteritidis* of Gaertner.

On testing the virulence of these organisms by intraperitoneal, and subcutaneous inoculation, I found that a single loopful of pure culture on agar of the bacillus C produced the death of guinea-pigs within 24 to 30 hours.   The only lesions found after death being a slight amount of peritonitis, intense hyperaemia of the small intestine, congestion of the lungs: there was no enlargement of the spleen.

[1] The streptococcus was not virulent.   Streptococci are very frequently present in cows' milk having absolutely no noxious properties.

Pure cultures of the bacillus were recovered from the blood of the heart.

A similar result was obtained by injecting 2 c.c. of a pure bouillon culture of the same bacillus under the skin of another guinea-pig.

Bacillus A proved much less virulent; an abscess formed at the seat of inoculation from which pure cultures of the *Bacillus coli* were obtained.

Bacillus B did not appear to be virulent.

In the light of the feeding experiments these results at first surprised me, but taking into consideration the state of the milk when it reached the laboratory, it seemed to me quite possible that the virulence of the milk had diminished as a result of the acid fermentation which had taken place during the 3 days it had been kept. That the milk had been virulent was sufficiently proved by the effects which it had produced in 7 human beings.

Further investigation has shown me that the most noxious among the samples of milk which I have studied had either an alkaline or an amphoteric reaction.

Faecal pollution the probable source of the 1894 Victoria Park epidemic.

The milk which had caused the Victoria Park outbreak contained therefore among other organisms a large number of bacilli indicating faecal pollution rather than an infectious disease of the cow's udder.

If the udder had been diseased it is difficult to understand why the outbreak occurred suddenly, and was caused by one or two milk deliveries alone.

In addition to this the cow which was suspected of having caused the outbreak was suffering from obvious mastitis, a lesion which as far as we know is not produced by the *Bacillus enteritidis*.

Non-tuberculous mastitis occurs frequently in milch cows, and I shall be able to show that there is no evidence that the milk of cows affected with such lesions is specially noxious.

It seemed to me easier to believe that milk-cans or other vessels had been infected on a certain occasion owing to the use of water heavily polluted with excreta of *a specially virulent kind,* and that the virulent bacteria so introduced had multiplied rapidly in the milk. There was also a distinct possibility of infection through dust, but this seemed to me less probable, considering the fact that the milk must have been exposed to very massive pollution. The state of things observed by

Dr Niven on the occasion of his visits to the farm, when they became known to me, confirmed me in my belief in the extraneous origin of the infection, a belief which I communicated to Dr Niven at the time.

Faecal pollution of milk a reasonable explanation of many cases of epidemic diarrhoea.

If in this case faecal pollution of the milk had produced an outbreak of disease indistinguishable from epidemic diarrhoea except by its time of occurrence and limited extent, it was reasonable to consider the possibility of a more intimate connection than had been generally admitted between epidemic diarrhoea and faecal contamination of milk.

Such contamination does frequently occur, as can easily be ascertained by inspection of badly-kept dairy farms, and by the microscopical and bacteriological examination of milk. It is even difficult to conceive how slight faecal pollution of cows' milk can entirely be prevented under any circumstance. Under ordinary conditions this contamination is apparently without serious effects, but when some animals in a herd are affected with intestinal inflammation, virulent bacilli must frequently escape from their bowel and infect more or less directly a portion of the milk, when that fluid is not collected under conditions of strict cleanliness. In summer these bacteria find in milk a suitable medium for rapid growth and multiplication, so that milk originally wholesome may thus be rapidly rendered noxious. On this supposition cows' milk would become most infectious at the time when epidemic diarrhoea is most prevalent, and would affect most severely infants of the age at which cows' milk becomes their chief food, *i.e.* at the time of weaning, which is the age at which mortality from infantile diarrhoea is greatest.

Working upon that hypothesis I began an investigation which I have carried on for the last 7 years, taking advantage of the large number of samples of milk which were sent to me for the detection of tubercle bacilli.

These samples were representative of the milk supplied to the inhabitants of large towns. Some were collected at railway stations on their arrival from the country, others were collected at the farms and represented the mixed milk of a small number of cows; finally many samples were collected from single cows, which, with few exceptions, had disease of the udder. In the latter case the milk was always collected in sterilized bottles by a veterinary surgeon, who was directed to take every precaution to prevent contamination of the milk by dirt

of any kind. By means of these samples I thought that it would be possible to recognise whether bacilli similar to those which had produced the Victoria Park outbreak were frequently present in the milk supplied to the public. The lesions produced in guinea-pigs inoculated with it would, I thought, allow me to detect their presence. It would also be easy to recognise the influence of disease of the udder, and of the keeping of milk in warm and cold weather for various lengths of time.

Examination of cows' milk (as supplied to towns) for the detection of bacteria capable of producing infection of a septic character.

Putting aside a number of preliminary examinations which had to be made for the purpose of determining the course to be followed in the investigation, nearly 2500 samples of milk have been examined in my laboratory since 1896.

During the years 1896 and 1897 I received many samples from a distance, mostly from Liverpool, these were sent in sterilized bottles, no precaution being taken to keep the temperature of the milk down. Specimens collected in Manchester under my direction were usually brought to the laboratory by hand, immediately after collection, either at the railway station or at the farm. As I have already explained, milk from diseased cows was collected by a veterinary surgeon, the cow being milked direct into sterilized bottles which I supplied. Having satisfied myself that milk coming from a distance, without precaution being taken to keep it cool, was infectious in a large proportion of cases, I refused at the beginning of 1898 to examine any more specimens unless they were collected in sterilized bottles, and packed in refrigerating boxes which I provided for the purpose. Since that time I have noticed a considerable improvement, and although many of the samples have been sent to me from distant places, including a town over 190 miles distant from Manchester, I have had much fewer cases of infection than used to be the case when unrefrigerated milk was forwarded from towns in the neighbourhood of Manchester.

This is shown by the following figures :

TABLE I. (See Diagram I, p. 92.)

Mortality from all causes occurring in guinea-pigs inoculated with cows' milk from 1896 to 1901 inclusive.

A. *Unrefrigerated mixed or unmixed milk examined during the years 1896 and 1897.*

Year	No. of samples	No. of samples causing the death of 2 animals inoculated in less than 10 days		No. of samples causing the death of 1 of the inoculated animals in less than 3 days		Total
1896–97	148	5	3·3 %	11	7·4 %	10·7 %

B. *Refrigerated mixed or unmixed milk examined from 1898 to 1901.*

1898	111	0	0 %	3	2·7 %	2·7 %
1899	175	1	0·57 %	1	0·57 %	1·14 %
1900	802	4	0·50 %	25	3·1 %	3·60 %
1901	694	1	0·14 %	8	1·1 %	1·24 %

N.B. The number of samples of milk coming from a great distance has gradually increased from 1896 to 1901.

The inference to be drawn from these gross results is clear: a certain proportion of the samples of milk contained bacteria which under favourable circumstances gave to the milk noxious properties. The development of these noxious properties could be checked in a large proportion of cases by preventing the growth of these bacteria. The difference between refrigerated and non-refrigerated milk would have been very much greater *if the milk had invariably been cooled at the farm immediately after the milking of the cows.* The mortality would have been diminished still further in the last three years if the veterinary surgeons collecting milk at farms at a distance from Manchester had been able to carry refrigerators with them; owing however to the number of specimens they had to collect in a single round they found it inconvenient to carry a refrigerator about with them, and all that could be done was to bring the samples as rapidly as possible to the laboratory where they were dealt with without further delay. The rise of mortality which occurred in 1900 was in great part due to the veterinary inspector getting in the habit of keeping a certain number of samples overnight before delivering them to the laboratory; an accident which I did not discover at once.

To find out whether the infectious bacteria present in the milk were chiefly derived from diseased udders or from extraneous contaminations, it is necessary to compare the effects produced by mixed

milk generally collected at railway stations, with those produced by the milk obtained from single cows affected with disease of the udder.

The following tables explain themselves.

Table II.

148 samples of milk examined before 1898. Generally kept for more than 24 hours. No precaution being taken to keep the temperature of the milk low by artificial means.

	Mixed milk collected at railway stations or town dairies	Milk collected direct from the udder of diseased cows. The milk being collected in sterilized vessels
	Per cent.	Per cent.
Milk producing no marked noxious effect	37·74	45·76
Milk producing chronic infection (non-tuberculous) not fatal	37·74	29·12
Milk producing acute infection rapidly fatal	17·76	0
Milk producing tuberculosis	6·66	24·96
Totals	99·90	99·84

Table II *a*. (See Diagram II, p. 92.)

By the exclusion of tuberculous milk the following percentages are obtained.

	Mixed milk	Unmixed milk
	Per cent.	Per cent.
Milk producing no marked noxious effect	40·38	60·5
Milk producing chronic infection not fatal	40·38	38·5
Milk producing acute infection rapidly fatal	19	—
	99·76	99·0

From these results it is obvious that the fatal infection produced in as many as 19·2 % of the animals inoculated with non-tuberculous mixed milk could be attributed to disease of the cow's udder *in a very few if any of the cases.* In fact in this first set of observations there was not a single case of general rapidly fatal infection produced by milk obtained from diseased udders.

TABLE III.

500 *samples of milk examined during the year* 1900 *for the Manchester Corporation. All these samples were collected in bottles sterilized in the laboratory, and were generally examined in less than* 10 *hours after the time of collection or refrigerated when kept longer. The mixed milk coming from country farms was not refrigerated during the transit by train between the farm and town, and the unmixed milk collected by the veterinary inspector was also not refrigerated during the transit from the farm to the laboratory. The samples of mixed and unmixed milk were generally obtained from the same farms at short intervals.*

	Mixed milk collected at railway stations or dairy farms 357 samples	Unmixed milk of diseased cows with diseased udder 143 samples
	Per cent.	Per cent.
Milk producing no marked noxious effect	67·2	62·2
Milk producing chronic infection not fatal	18·7	13·9
Milk producing acute infection rapidly fatal	1·68	1·39
Milk producing tuberculosis	12·3	22·3
	99·88	99·89

TABLE III a.   (See Diagram III, p. 93.)

By the exclusion of tuberculous milk there remain 424 *samples of non-tuberculous milk.*

	Mixed milk collected at railway stations or dairy farms 313 samples	Unmixed milk of diseased cows with diseased udder 111 samples
	Per cent.	Per cent.
Milk producing no marked noxious effect	76·6	80·1
Milk producing chronic infection not fatal	21·4	18·0
Milk producing acute infection rapidly fatal	1·9	1·8
	99·9	99·9

N.B.   It is to be noticed that when conditions of keeping and temperature are equalised, mixed and unmixed milk have much the same properties.

To find out the conditions which favoured the development of infectious properties I have prepared the following tables[1], which include only specimens which had been collected before special precautions were taken to prevent bacterial multiplication.

TABLE IV. (See Diagram IV, p. 93.)

1. *Mixed milk coming from a* DISTANCE OF OVER 40 MILES, *and generally kept for from* 24 TO 60 HOURS, *and even longer in a few cases. (Tuberculous samples excluded.)*

Mean temperature Fahr. in the shade (Manchester) during time the specimens were kept	Specimens producing no noxious effects	Noxious specimens	Totals	Percentage of good specimens
30° to 35°	7	5	12	58·0
35° to 40°	7	11	18	38·5
40° to 45°	2	3	5	40·0
45° to 50°	1	4	5	20·0
50° to 55°	—	—	—	—
55° to 60°	0	2	2	0·0
	17	25	42	39·0

TABLE V. (See Diagram V, p. 94.)

2. *Mixed milk coming from a short* DISTANCE (GENERALLY UNDER 20 MILES), *most of them kept for* LESS THAN 10 HOURS (*with the exception of 5 out of the 7 bad specimens, and 4 out of the 22 good specimens which had been kept somewhat longer. Tuberculous samples excluded*).

Mean temperature Fahr. in the shade (Manchester) during time the specimens were kept	Specimens producing no noxious effects	Noxious specimens	Totals	Percentage of good specimens
50° to 55°	1	0	1	100·0
55° to 60°	8	1	9	88·8
60° to 65°	11	4	15	75·2
65° to 70°	—	—	—	—
70° to 75°	2	2	4	50·0
	22	7	29	75·68

[1] For details see an article which I published in 1897, "The examination of cow's milk for the detection of pathogenic properties," *Journal of Comparative Pathology and Therapeutics*, 1897.

TABLE VI. (See Diagram VI, p. 94.)

3. UNMIXED MILKS *kept for various lengths of time, but* COLLECTED FROM THE UDDER *in sterilized vessels.* (*Tuberculous samples excluded.*)

Mean temperature Fahr. in the shade (Manchester) during time the specimens were kept	Specimens producing no noxious effects	Noxious specimens	Totals	Percentage of good specimens
35° to 40°	6	0	6	100·0
40° to 45°	3	2	5	60·0
45° to 50°	5	2	7	71·5
50° to 55°	—	—	—	—
55° to 60°	—	—	—	—
60° to 65°	0	3	3	0·0
	14	7	21	67·2

The influence of time is well shown by the number of specimens remaining good even at a high temperature when the milk had been kept only half-a-day.

On the other hand, the influence of temperature is still more evident, for in every category the number of good specimens is almost inversely proportional to the height of the temperature. Still it is important to keep the two factors of time and temperature in mind. *What is produced in a few hours in summer may occur also in winter when the milk has been kept a long time.*

When the clear relation existing between time of keeping plus temperature and the noxious properties of a certain number of samples of milk, is contrasted with the ambiguous results obtained when an attempt is made to connect these noxious properties with disease of the udder (tuberculosis being excluded), it is difficult not to feel convinced that infection of the milk outside the udder, and the conditions under which milk is kept, are the most important factors causing it to acquire infective properties.

I have found a close resemblance between the lesions produced in guinea-pigs by the inoculation of milk which had given rise to limited outbreaks of summer diarrhoea, and that produced in the same kind of animals by the injection of many samples of mixed milks obtained on the market. In both cases I have very frequently isolated from the blood or local lesions of acutely infected guinea-pigs bacilli belonging to the colon bacillus group. These bacilli were often the only organisms found, especially when the animals were killed before the termination of their illness. In about 90 % of the cases of fatal septicaemia due to

milk inoculations of guinea-pigs I have been able to isolate from the blood bacilli resembling closely, or identical with, those obtained twice from milk causing intense diarrhoea in children and adults. They have a similar resemblance to bacilli which I have isolated from the organs of patients suffering from cholera nostras of doubtful origin, and from the organs of several patients suffering clearly from food poisoning; I have also found the same kind of bacilli in cheese which had given rise to severe attacks of food poisoning. These microbes retain their virulent properties even after being cultivated for several generations outside the body. They multiply with extraordinary rapidity at Summer temperature. They resemble the *Bacillus coli communis* or the bacilli of the colon group in their pathogenic action, their mode of growth on gelatine, agar, potato, milk, lactose agar, glucose gelatine, and in their size, shape, and motility, allowance being made for the variability of the bacilli of this group.

I have not found any reason to alter these views which I published almost in the same terms in 1897. Since I first expressed, in 1894, my belief in the connection between the infections of food by colon bacilli and Summer diarrhoea, Dr Klein's work on the *Bacillus enteritidis sporogenes* has appeared. *Klein's bacillus, which has no relation to the colon group*, would apparently have to milk the same relation as the bacilli of the colon group, being introduced into that fluid through faecal pollution. All I have said in favour of the view that bacilli of the colon group are essential factors in the production of Summer diarrhoea might be said of the *Bacillus enteritidis sporogenes*. The chief difficulty which I find in accepting Dr Klein's theory is, that out of the large number of samples of milk I have examined by inoculation, I have not found more than two samples per 1000 producing lesions which could be attributed to the *Bacillus enteritidis sporogenes*, whilst during the same time I have found that from 100 to 200 or more per 1000 samples according to the season or length of keeping, were capable of causing infection attributable to a bacillus of the colon group. Streptococci did not seem to be responsible for a greater proportion of cases than the *Bacillus enteritidis sporogenes*[1].

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[1] It is somewhat difficult to estimate the share taken by staphylococci and streptococci in the production of lesions following milk inoculations, they are found frequently enough in animals dying more than 10 days after inoculation, and they may in such cases be associated or not with bacilli of the colon group, but they are not usually found in animals dying from acute septicaemia. It is probable that in many instances their presence is due to secondary infection.

*Comparison of Epidemiological Data and of Bacteriological Data
resulting from the examination of Cows' Milk.*

A consideration of the facts brought to light by the valuable contributions of Dr Ogle, Dr Ballard, Dr Newsholme and others, impresses me with the belief that temperature is on the whole the most important of the factors determining the rate of mortality from epidemic diarrhoea.

Dr Newsholme's able and exhaustive " Contribution to the Study of Epidemic Diarrhoea" (*Public Health*, x. p. 139, 1899—1900) leaves no doubt upon this subject. The influence of other conditions is fully discussed in Dr Newsholme's address, but although those conditions have undoubtedly to be considered, they are in my opinion of secondary importance with regard to their relation to case incidence.

Dr Newsholme concludes that the disease is due to a particulate poison which infects the air, and is swallowed, most commonly with food, especially milk. In this he agrees with Ballard, and with regard to milk he is also in agreement with the position I had previously taken.

In discussing the influence of milk, however, Dr Newsholme does not attach so much importance as I had done to infection at the cowshed, either through dirty udders, dirty hands, or dirty vessels. He is of opinion that milk is probably generally infected during storage at home in places where it is exposed to pollution by infective dust.

My results do not exclude infection at the home of the consumer, or during transit from the farm, but they indicate that infection at the farm, or through vessels infected at the farm and used by the farmer for the storage and carriage of milk, must be of paramount importance. *None of the milk I have examined had been exposed to any influence attributable to a consumer's home.* It will be noticed that a large proportion of the samples of milk obtained from cans at railway stations or at the farms *is already infectious before it reaches the consumer*; also that the degree of noxiousness acquired through infection is proportional to the length of time the milk has been kept, and the temperature which it has been exposed to, before it reaches the consumer. It seems to me that the most dangerous form of infection which takes place at the consumer's home is that which results from the placing of fresh milk in vessels or feeding-bottles which have previously contained infected milk, and which have not been sufficiently cleansed or sterilized

afterwards. It is therefore obvious that long keeping and high temperature are the two important factors which determine whether a sample of infected milk will contain a sufficient quantity of bacteria or bacterial products to produce infection. The conditions necessary for this development of noxious properties are generally more easily attained during transit from the farm in hot railway vans, than after the milk has reached the home of the consumer. The same factors are of course at work at the last place, but usually the time is shorter and the temperature lower. Infection, long keeping, and high temperature are all compatible with many town cellars or pantries, but taking all the facts I have collected into consideration it appears to me that infection at the farm or through milk-cans or other vessels is by far the most important factor. According to this view the explanation of a large proportion of cases of infantile diarrhoea becomes an easy matter; infection through cows' milk explains readily the special incidence of the disease and the high mortality of infants between the 3rd and the 12th month. The difficulty of obtaining fresh milk in the centre of poor populous districts would also explain why so much of the milk consumed by poor children of large towns is infectious, for such milk has often to travel over considerable distances under very unfavourable conditions. The better classes are generally supplied with fresher milk than the poorer classes[1]. A rapidly multiplying bacillus when once introduced into a feeding-bottle which is not frequently sterilized will render the bottle itself a source of infection to all the milk subsequently placed in that bottle. Such an accident is more likely to occur in homes which are unclean, where the mother who works out of doors has to leave her last born to the care of a previously born child or of strangers.

The epidemic diarrhoea of adults is not so easily explained, because milk does not form such an important part of the regular food of the adult population, and also because the adult is much less liable to suffer from the consumption of contaminated food. It is however to be remembered that milk is not the only food which is exposed to faecal contamination, meat, fish, molluscs, vegetables, fruit, fresh or preserved, are all liable to pollution, specially when prepared for consumption in dirty premises.

The study of outbreaks like the one I have recently investigated in Derby has convinced me that specific faecal pollution, generally at the time of manufacture, of various prepared articles of food, is responsible for a large number of epidemics of food poisoning.

[1] Condensed milk *is not*, as is often supposed, *sterilized* in the process of manufacture; it often contains a large number of bacteria of various kinds and a variable amount of dirt.

Some remarks about the Derby outbreak of food poisoning.

The pork-pies which caused the Derby outbreak contained a large number of bacilli having characters closely allied to those of the *Bacillus enteritidis* of Gaertner, and of various bacilli which I had isolated from pathogenic milk during the last eight years. A careful examination of the pies led me to the conclusion that the meat of the pies had been infected by coming in contact with some infectious faecal matter. An inspection of the premises where the pies had been prepared convinced me that such an infection was not only possible but probable, and I was even able to indicate the part of the premises where the infection had occurred. My conclusions were entirely confirmed by subsequent enquiries conducted by Dr Howarth, who found that the meat which had been used in the preparation of the most noxious batch of pies had been exposed to special faecal pollution, owing to the cleaning of apparently diseased bowels in a room where the pie meat was left in uncovered vessels.

A specially interesting feature of the Derby outbreak is, that although a large number of persons (at least 130) fell ill in Derby, none died in that town during the epidemic (one fatal case occurred a considerable time afterwards from complications which were possibly, though not necessarily, the result of the infection).

Of the cases (about 90) occurring outside the borough, some at a considerable distance, at least four ended fatally. (I was able to prove the presence of the *Bacillus enteritidis Derbiensis*[1] in the organs of two of these fatal cases; I had not an opportunity to examine the organs of the others.)

Thus the pies had increased in virulence on being kept, just as milk does when it is sent from a distance to a town, and is not consumed soon after collection.

Is there a specific bacillus capable of causing Epidemic Diarrhoea?

I have previously stated that from the milk which caused the Victoria Park epidemic I isolated several bacilli belonging to the colon group, one of which was very virulent, causing rapid septicaemia when injected into the peritoneum, and somewhat less rapid septicaemia when injected under the skin of guinea-pigs. The chief lesions observed after

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[1] Report on the Recent Outbreak of Food Poisoning in Derby. Derby (Richard Keene, Limited), 1902.

death were congestion of the small intestine, which contained only
mucus, more or less bile stained, great congestion of the lungs; the
spleen was small or slightly enlarged. From the blood of the heart and
of all the organs the bacillus could easily be recovered. This bacillus
had characters resembling closely those of *B. enteritidis* (Gaertner).
The lesions observed in about 90 % of the guinea-pigs which succumbed
rapidly to the inoculation of milk supplied to towns, were similar to the
above, with the difference *in a few cases* that the spleen was enlarged,
or that peritonitis was more marked. The bacillus isolated from the
blood of these animals resembled more or less closely the bacilli
obtained from the milk which had caused the Victoria Park epidemic.
In four cases out of ten a pure culture of a bacillus resembling Gaertner's
bacillus was obtained; in the other cases I found a mixture of bacilli
belonging to the colon group, some approaching the type of Gaertner's
bacillus, others resembling more the *Bacillus coli* (Escherich).

Animals fed on the pies which had caused the Derby epidemic
presented after death lesions similar to those which I have described
above (according to the duration of the experimental illness the spleen
was either small or enlarged), and the bacillus isolated from their blood
had characters allied to those of Gaertner's bacillus.

From the blood of two patients who had died from the consumption
of the Derby pies the same bacillus as that obtained from the organs of
guinea-pigs fed on the pies was isolated, but it was accompanied by
bacilli identical with or resembling *Bacillus coli communis*.

All these facts put together, as well as many of those which have
been previously recorded, indicate that the infectious properties which
food acquires frequently in summer, and which give rise to the *ordinary
or common type of epidemic diarrhoea* are generally due to bacilli
belonging to the colon group of bacilli of which the *B. coli communis*
(Escherich) and the *B. enteritidis* (Gaertner) are probably two extreme
types. I have come also to the conclusion that the varieties of these bacilli
which are the most important sources of infection are those which
resemble the bacillus of Gaertner, and which therefore produce *no
permanent acidity, coagulation, or distinct smell when grown in milk*.
Very few of the infectious samples of milk which I have examined
during the last five years had a distinct acid reaction, so that *absence of
acidity and marked smell in milk is not, as generally believed, an index
of safety*.

It is probable that the most dangerous kind of faecal infection is
that produced by matter containing bacilli resembling the Gaertner's

bacillus. *Such an infection is probably connected with the existence of an infectious diarrhoeal disease liable to occur in the lower animals as well as in man.* I have not however been able to satisfy myself entirely regarding this point.

It is certain however that bacilli presenting the characters of the ordinary *B. coli communis* are seldom capable of producing such a rapid infection as that produced by the *B. enteritidis,* or by closely allied bacilli such as the *B. enteritidis Derbiensis.* I hope that an investigation which I am at present conducting with the cooperation of Dr A. Sellers will allow me to speak before long more definitely regarding the properties of this group of bacilli.

General Conclusions.

1.   *Epidemic diarrhoea of the common type occurring in this country* is apparently in the great majority of instances the result of infection of food by bacilli belonging to the colon group of bacilli which are present at times in faecal matter.

2.   It appears that this infection of food does not generally lead to serious consequences unless the infection is massive from the first, or the food is kept for a sufficient length of time and under conditions of temperature favouring the multiplication of these bacilli.

3.   Milk which is the most common cause of epidemic diarrhoea in infants is frequently infected at the farm or (through vessels) in transit.

4.   Other foods than milk are also liable to infection before they reach the consumer.

5.   Of the bacilli of the colon group, which are capable of rendering the milk infectious, those which do not produce a large amount of acid, and do not coagulate milk are the most virulent, and are probably the essential cause of epidemic diarrhoea.

Preventive measures against Epidemic Diarrhoea.

The preventive measures which seem to me to be most clearly indicated by the above conclusions are the following.

1°.   Measures securing *cleanness* of cows, dairy hands, cowsheds, milk vessels etc.[1]   Similar measures are also needed with regard to persons or

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[1] See in this connection a paper by W. H. Park (1901) " The great Contamination of the Milk of Cities. Can it be lessened by the action of Health Authorities ? " *Journ. of Hygiene,* Vol. I. p. 391.   *Ed.*

things coming in contact with any other article of food, manufactured or not.

This I think is generally admitted to be a most important element of good sanitation, but absolute cleanness is most difficult to obtain if not practically impossible. Infection must therefore occur now and again.

2°. To guard against the worst effects of accidental faecal infection the food should be *consumed fresh*, when possible.

3°. When the food cannot be consumed fresh it should be *refrigerated, i.e.* kept at a temperature below 4° C.

4°. Where the food cannot be eaten fresh or refrigerated whilst it is kept, it should be thoroughly *sterilized* by heat (*i.e.* by thorough cooking).

N.B. The above precautions refer only to epidemic diarrhoea; other forms of infection may be dealt with in other ways, thus the only safeguard against tuberculous milk is boiling or entire exclusion of tuberculous cattle from our herds.

DIAGRAM I.   (See Table I.)

Effects of refrigeration on the lethal properties of milk.   Percentage of samples examined which have proved fatal to 2 guinea-pigs in less than 10 days.

No. of fatal samples %

No. of fatal samples %

Black = death.

1896-7   1898   1899   1900   1901

No Refrigeration   Refrigeration
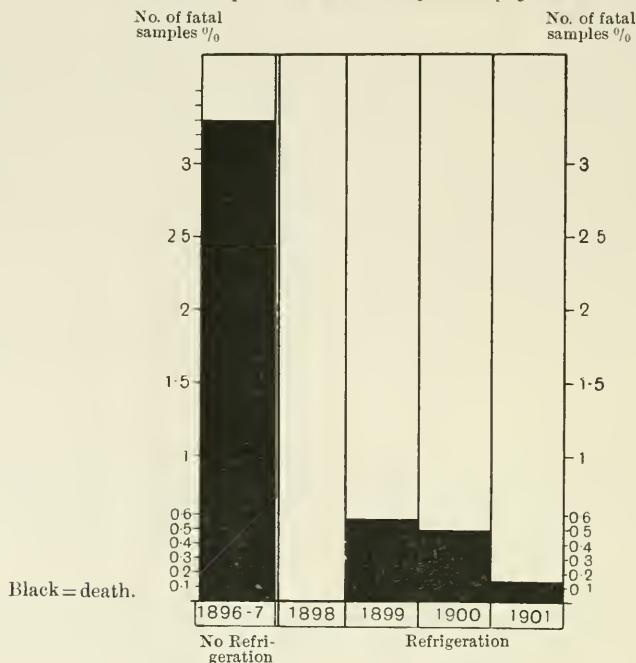
DIAGRAM II.   (See Table II *a*.)

Showing the proportion of noxious samples of milk per centum of samples tested by inoculation.   (Tuberculous samples excluded.)

No. of * samples

Unmixed milk from diseased cows with diseased udder

Mixed milk collected at railway stations or at farms

No. of * samples

Black = death
Hatching = infection not fatal
Dots = no evidence of infection

Unmixed milk   Mixed milk
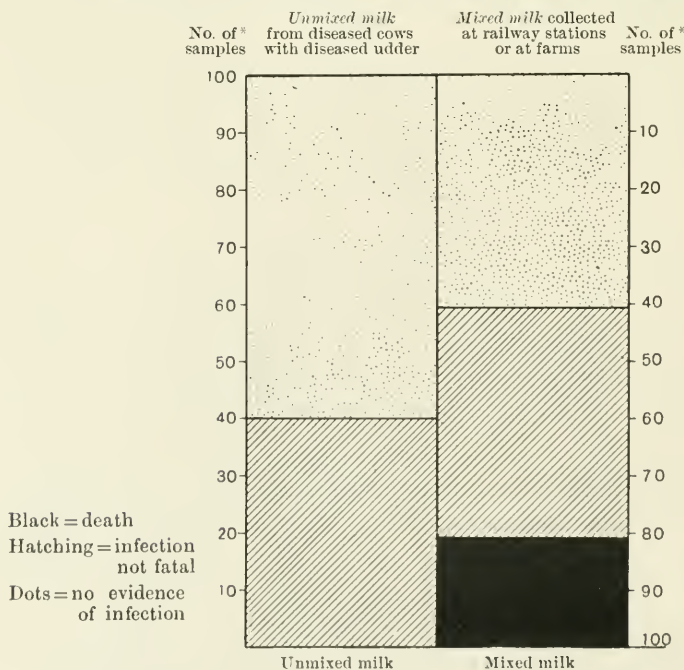
* The figures on the right side of the diagram give the percentage of good specimens, those on the left the percentage of bad specimens.

DIAGRAM III. (See Table III *a*.)

*Showing the proportion of noxious samples of milk per centum of
samples tested by inoculation during the year 1900.*
*500 Manchester samples only, most samples examined in less
than 10 hours after collection.*

Unmixed milk	Mixed milk collected
from diseased cows	at railway stations
with diseased udder	and at farms

Black = death.   Hatching = infection not fatal.   Dots = no infection.
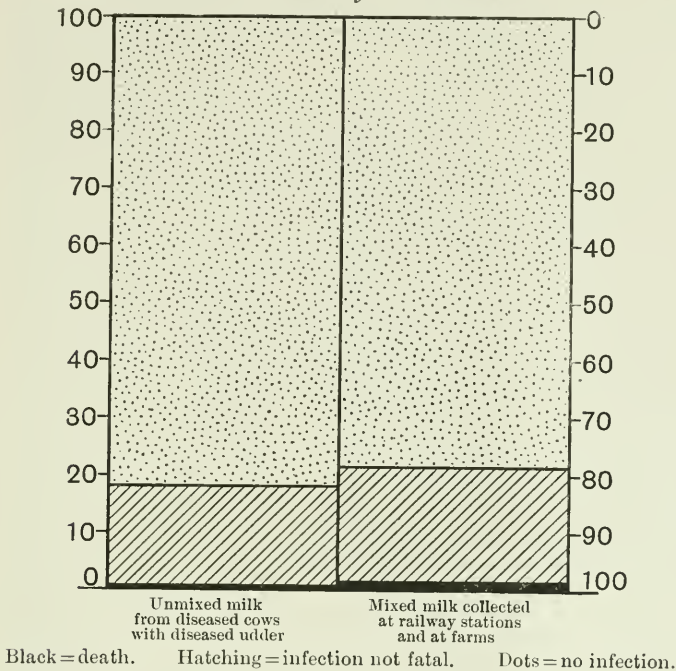
DIAGRAM IV. (See Table IV.)

Effects of **temperature** *upon the noxious properties of milk sent to
Manchester from a distance of* **over 40 miles** *and kept for* **24 to
60 hours**. (*Tuberculous samples excluded.*)

Hatching = infection
  including fatal
  cases.

Dots = no evidence of
  infection.

Temperature   30°   35°   40°   45°   50°   55°   60° F
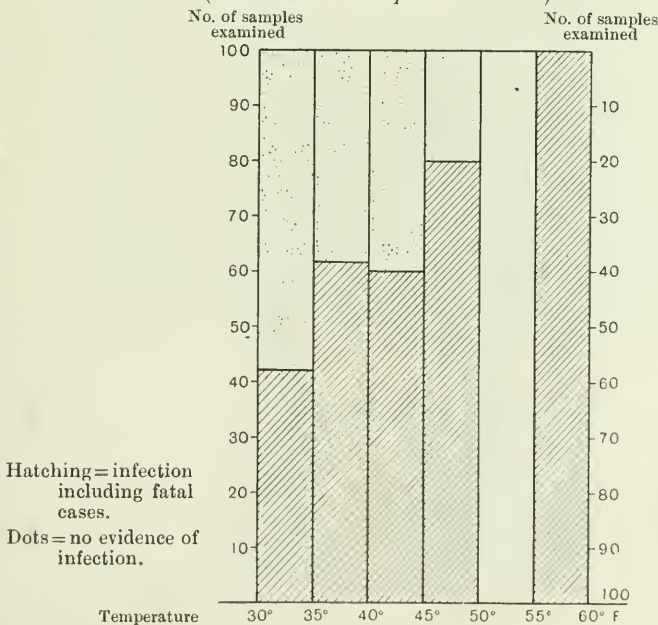
DIAGRAM V. (See Table V.)

Effects of temperature *upon the noxious properties of milk sent to Manchester from a distance of* less than 20 miles, *and generally kept for* less than 10 hours. (*Tuberculous milk excluded.*)

No. of samples examined ... No. of samples examined

Hatching = infection including fatal cases.

Dots = no evidence of infection.

Temperature   50°   55°   60°   65°   70°   75° F

DIAGRAM VI.   (See Table VI.)

Effects of temperature *upon samples of unmixed milk from* diseased cows, *collected in sterilized bottles and with care, but* kept for various lengths of time. (*Tuberculous milk excluded.*)

No. of samples ... No. of samples

Hatching = infection.

Dots = no evidence of infection.

Temperature   35°   40°   45°   50°   55°   60°   65° F

J. S. Barrow, Camborne   Copyrighted

Gig at 302-Fathom Level, Dolcoath.

AN OUTBREAK OF ANKYLOSTOMIASIS IN ENGLAND. No. I.

(Plates I—V. and One Figure.)

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Senior Demy of Magdalen College, Oxford;

and J. S. HALDANE, M.D., F.R.S.,
Fellow of New College, Oxford.

CONTENTS.

THE outbreak which is the subject of the present paper was discovered in the course of an enquiry which is at present being made on the request of the Home Secretary by Mr J. S. Martin, Inspector of Mines, and one of us, into the ventilation of Cornish mines. A number of cases of anaemia had occurred among the miners employed at Dolcoath Mine, Cornwall; and as the anaemia was generally attributed to some defect in the ventilation, this mine was among the first visited. On examination into the state of the mine, the symptoms of the affected men, and the history of the outbreak, it appeared that the anaemia was almost certainly caused by ankylostomiasis. A more detailed enquiry, in which we have both been engaged, established beyond all doubt the nature of the disease, and has furnished the opportunity of investigating a number of points bearing on the nature and method of spread of ankylostomiasis. A short report on the outbreak has already appeared[1]. We are indebted to Dr S. G. Scott,

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[1] Haldane, "Report to the Home Secretary on an Outbreak of Ankylostomiasis in a Cornish Mine," *Parliamentary Paper*, 1903.

who has also been engaged in the Home Office Enquiry, for a number of valuable notes as to blood-examinations, and many other details.

As is well known, ankylostomiasis is one of the commonest diseases in tropical and sub-tropical countries. Its main symptoms are those of anaemia; and it is due to the presence in the upper part of the small intestine of numerous small worms of the species most commonly known as *Ankylostoma duodenale.* The worm itself was first described in Italy by Dubini[1] in 1838, but its definite association with anaemia was discovered by Griesinger[2], who showed that the disease known as Egyptian Chlorosis is due to its presence. A few years later Wucherer showed that the anaemia common in Brazil is ankylostomiasis; and ankylostomiasis has since been identified in many other tropical and sub-tropical countries as a common, wide-spread, and very troublesome disease. About twenty years ago there occurred a very serious outbreak of anaemia among the men engaged in the construction of the St Gothard tunnel. This was shown by Perroncito and others to be ankylostomiasis. The disease was more fully studied, and the present methods of treatment by large doses of extract of male fern or thymol were introduced. In recent years the disease has spread in French, Belgian, German, and Austrian collieries, causing much trouble.

The worm does not reproduce itself within the intestine, but the female produces an enormous number of ova, which pass out in the faeces, and under suitable conditions of temperature etc. develope into larval worms. According to some observations there is a free sexual form of the worm outside the body, but it is most generally believed that this is not the case, and that infection is conveyed by swallowing the larval worms. It has recently been shown by Looss[3] that the young worms are capable of penetrating into the hair-follicles and producing a local dermatitis. The same observer believes that the young worms may reach the intestine by passing onwards from the hair-follicles.

The Conditions met with in Dolcoath Mine.

As the occurrence of ankylostomiasis in an English mine evidently depends upon the special conditions underground, some account must be given here of these conditions as they exist at Dolcoath and in other similar mines.

[1] *Annali Univ. di Medicina,* 1843.

[2] *Archiv f. physiol. Heilkunde,* Vol. xiii., 1854, p. 555.

[3] *Centralbl. f. Bakt. u. Parasitol.* 1898, Vol. xxiv., pp. 483—488; 1901, Vol. xxix., pp. 733—739.

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In 170-Fathom Level, East Pool Mine.

PLATE III

J. N. Burrow Camborne

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Croust-time in a Cornish Mine.

The Cornish mines, including Dolcoath, are all metalliferous, by far the most important metal extracted being tin. The ore occurs in lodes, which run downwards through the "killas" (an argillaceous schist) and granite. The workings follow the lodes downwards, the lode being extracted wherever it is rich enough to repay the expense, and left where this is not the case. Fig. 1 (p. 136) shows a vertical section of the Dolcoath Main lode, which runs down nearly vertically through the killas and granite. The depths are indicated in fathoms (1 fathom = 6 feet or 1·83 metres). It will be seen that copper ore occurred in the upper part of the lode, and was formerly mined. The present workings are mostly at a depth of over 2000 feet and have reached nearly 3000 feet. Only tin ore is now obtained.

The ore is wound up through several shafts in iron boxes known as "skips," which run on wheels wherever the shaft is not vertical. Somewhat similar iron boxes known as "gigs" are used for the conveyance of men to and from their work (see Plate I.)[1]. There are also ladder-ways at the sides of the shafts, and at many points throughout the workings (Plates I. and II.), to enable the men to pass from level to level. In working the lode the shaft is first sunk, and levels driven horizontally outwards from it (see Figure 1). The latter are then connected at intervals with the level above by "winzes" driven upwards or downwards through the lode, by which means adequate ventilation of the levels is secured. The portions of the lode, or "stopes," thus blocked out are then gradually removed, the ore being shot down into the level below and removed to the shaft on small wagons running on rails and pushed by hand. In driving the levels and "rises" passing upwards from them, rock-drills driven by compressed air are commonly used for boring the holes which receive the explosive (usually dynamite). On the stopes hand-drills are used. The space left after the lode has been extracted is known as a "gunnis," and may extend upwards or downwards for hundreds of feet. When a level crosses a "gunnis" the roadway is supported on timber.

The general ventilation of a Cornish mine is produced, almost invariably, by "natural" means only. Large fans or furnaces, as in coal-mines, are not employed, nor would it be possible to employ them unless the shafts were closed laterally so as to prevent short-circuiting between downcast and upcast shafts. The air-currents down the downcast, and up the upcast shafts depend on the fact that the

[1] Plates I. II. and III. are from photographs taken by magnesium light by Mr I. S. Burrow, photographer, Camborne, and reproduced by his permission.

temperature below is usually much higher than on the surface, and that even when, in warm weather, the difference of temperature would not by itself suffice to produce a current, the temperature in any shaft which has been previously acting as a downcast will always be lower, at the same levels, than in the corresponding upcast shaft. The general course of the air-currents in Dolcoath mine is indicated by the arrows on the section. It will be seen that roughly speaking the air passes down by the shafts at each end of the section of lode worked, and passes up by the shafts in the middle. The temperature is consequently a good deal lower in the Eastern and Western (Stray Parks) shafts than in the middle shafts—a fact which, as will be seen below, was probably of considerable significance in relation to the spread of ankylostomiasis among the miners.

To ascertain the amount of impurity in the air of the mine samples were taken at the points marked A to H on the section. These samples were collected on October 16th 1902 in stoppered bottles by the method described in this *Journal*, Vol. II. p. 416. The analyses were made by the method described by one of us in the *Journal of Physiology*, Vol. XXII. p. 465, 1898, the gas-burette being that shown at Fig. 5 of the same paper. The results are given in Table I.

TABLE I.

Air Analyses at Dolcoath Mine.

	Oxygen per cent.	CO_2 per cent.	Oxygen diminished per cent.	CO_2 increase per cent.
Outside air (was perfectly pure).	20·94	·03	0·00	0·00
A. 302 fathoms level in Eastern (downcast) Shaft.	20·94	·03	0·00	0·00
B. 302 fathoms level at end, cross-cut in granite, air blown in.	20·82	·08	0·12	0·05
C. 375 level, North Lode, at stoping in open place.	20·79	{ ·110 ·115 }	0·15	0·08
D. 412 level, large open gunnis (dry 77°, wet 76°).	20·85	·095	0·09	0·065
E. 440 level, at New Bridge (dry 77°, wet 76°).	20·83	·09	0·11	0·06
F. 470 level, rise in crosscut north of lode, drill idle.	{ 20·67 20·66	·22 ·23 }	0·275	0·225
G. 455 level, bottom of Engine (upcast) Shaft (dry 79°, wet 78°).	20·85	·095	0·09	0·065
H. 375 level, taken from gig in Engine Shaft.	20·79	{ ·110 ·115 }	0·14	0·082

Samples B and H were examined with an incandescent platinum spiral for CH_4, CO, etc., but no traces were found.

It will be seen that the air in the shafts, open levels, and stopes was on the whole surprisingly pure, chemically speaking. The anaemia among the miners could evidently have nothing to do with impurities in the air. On the day when the samples were taken it was found that about 14000 cubic feet (400 cubic metres) of air per minute were passing down the Eastern shaft at A.

The impurities found in the air have several sources—(1) human respiration, (2) combustion of candles, (3) combustion of dynamite, (4) decay of timber, and (5) chemical changes occurring in the lode and surrounding strata. The number of men employed, and the weight of candles and dynamite burnt, between the Eastern (downcast) and Engine (upcast) shafts, would scarcely account for more than about a third of the impurity found in the upcast air; and probably the greater part of it is due to chemical change in the lode and surrounding strata. In certain unventilated levels, where work had been suspended, we obtained clear evidence of the influence of chemical changes in the lode on the purity of the air. Thus in one such level, at the point marked K on the section, which was very hot and dry, the air at the point where a sample was collected caused panting (due to the carbonic acid), and a tallow candle would only burn when held horizontally, with the wick well spread out. The sample consisted of:

> Oxygen $18 \cdot 00$.
> Carbonic Acid $3 \cdot 18$.
> Nitrogen $78 \cdot 82$.

On examining the roof numerous crystals of calcium sulphate were found, along with hydrated oxide of iron. As iron pyrites (FeS_2) was also present in the lode, and water containing carbonate of lime in solution, it was evident that a chemical process represented probably by the following equations was occurring:

$$4FeS_2 + 15O_2 + 8H_2O = 2Fe_2O_3 + 8H_2SO_4, \text{ and}$$
$$8H_2SO_4 + 8CaO, H_2O, (CO_2)_2 = 8CaSO_4 + 16H_2O + 16CO_2.$$
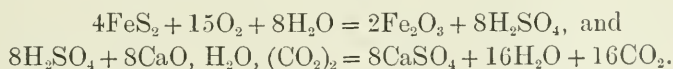
This process would yield air similar to that found, with a slightly greater quantity of carbonic acid than corresponded to the oxygen which had disappeared. It would seem from some of the analyses in Table I that at other parts of the mine carbonic acid may be reabsorbed by carbonate of lime to form bicarbonate, or to form carbonate of potash from the silicate contained in the granite, as often the increase in carbonic acid (at Dolcoath and other Cornish mines) is considerably less

than the diminution in oxygen. Similar oxidation processes often occur on a much larger scale in coal-mines[1].

The temperatures observed in the Eastern (downcast) and Engine (upcast) shafts, and at various points in the adjoining workings, are shown on the section. The highest temperature indicated (at the bottom of the upcast shaft) is 79° F. (26° C.), but in driving levels and rises from them to levels above, higher temperatures are experienced. A temperature of nearly 90° F. (32° C.) is not unusual at such places in Cornish mines.

There are several causes for the high underground temperature— (1) the heating of the air by compression as it descends the downcast shaft; (2) the higher natural temperature of the strata as depth increases; (3) the presence of men, and the combustion of candles and explosives; (4) heat-production due to oxidation in and about the lode. As the propagation of ankylostomiasis in mines depends to a large extent on temperature, a brief discussion of these various causes is desirable.

Heating of the air by increase of atmospheric pressure as it descends the downcast shaft is observed in all mines. This heating amounts to about $5\frac{1}{2}$° F. (3° C.) for every 1000 feet. As, however, the temperature of the air in descending is affected by the temperature of the shaft-walls, and by the amount of moisture taken up from the walls, the actual difference in temperature between the air at the top and the bottom will seldom correspond exactly to the difference stated. The actual temperature at the bottom seems to correspond approximately to the excess due to compression over the *mean* annual temperature on the surface. At Dolcoath the temperature at the 412 fathom level below adit (= 2500 feet below surface) was 68·5° F. (20·3° C.). As the mean annual temperature at Camborne is about 53° F. (11·7° C.) a temperature of 67° F. (19·5° C.) at this depth would be expected according to the above rule. A somewhat higher temperature is, however, to be expected in summer and autumn than in winter and spring. In an upcast shaft the air is always cooled by decompression. As, however, the air is nearly saturated with moisture at the bottom, mist is formed in the air on its way upwards, and a corresponding amount of latent heat liberated, so that the actual cooling of the air on its way upwards is not so great as the heating on the way downwards. The heating in the downcast shaft, with corresponding cooling and

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[1] Haldane and Meachem, *Trans. Inst. of Mining Engineers*, Vol. XVI. 1899.

deposition of moisture in the upcast, is the converse of the phenomena observed when wind blows over a range of high mountains (*e.g.* the Alps). The dangerous drying effect produced by the air in the intake roads of a deep colliery may be compared with the corresponding effects of the " Föhn " wind in Switzerland, while the mist and deposition of moisture always observed in an upcast shaft may be compared with the rain and snow deposited by wind in passing over a chain of mountains.

It will be seen from the section that the temperature in the workings at Dolcoath, as in other mines, is considerably higher than can be explained by the rise due to compression of the air. The difference is certainly due in part to the increased natural temperature of the strata, but there is no doubt that the influence of this factor has been greatly exaggerated, and that the limit of depth at which mining can, with intelligent precautions, be carried on without serious inconvenience from the heat, is very far from having been reached. A series of temperature observations was, for instance, made at Dolcoath for the Royal Commission on the Health of Metalliferous Miners, which reported in 1864[1]. Although at that time the depth reached by the workings was less by 1200 feet than at present, yet the temperatures observed (76° to 84° F.) near the bottom of the Engine Shaft were quite as high as those now met with at corresponding positions 1200 feet lower on the same shaft. According to the most reliable observations in bore-holes the average increase in the natural temperature of the strata is about 1° F. for every 70 feet, or 1° C. for every 126 feet, but the amount varies considerably at different places, and the workings of a mine may be either above or below the natural temperature of the strata, according as the ventilation varies in relation to the heat-production by oxidation[2]. Assuming that the temperature in Dolcoath mine away from the downcast shaft depended on the natural temperature of the strata, and that there was an increase of 1° F. for every 70 feet of depth, we should expect to find a temperature of 94° F. at the bottom level, which is 2850 feet below the surface, whereas the actual temperature is only about 79° F. (26° C.).

The difference may be partly due to a less rate of temperature-increase in the strata at Dolcoath than at most other places, but it is not necessary to assume this. In the paper just referred to it was shown that the heat-production from oxidation in the coal at Hamstead Colliery (as in many other collieries) was greatly in excess of the heat

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[1] See Section of Dolcoath Mine at page 14 of Appendix A ; also Appendix B, p. 298.

[2] Haldane and Meachem, *loc. cit.*

carried away by the ventilation-current. In consequence of this the temperature of the mine was rising progressively, and spontaneous fires in the coal were becoming more and more troublesome[1]. The heat-production, as compared with the heat carried away by the ventilation current, was calculated from the deficiency of oxygen in the upcast shaft as compared with the readings of dry and wet-bulb thermometers at the bottoms of the downcast and upcast shafts respectively. A similar calculation made for Dolcoath mine shows that the heat carried away by the ventilation-current is considerably in excess of the heat formed in the mine. The workings are therefore being on the whole progressively *cooled* by the ventilation-current, and their comparatively low temperature is thus easily explained. In headings &c. a high temperature must, however, be met with unless the local ventilation is sufficient to carry off the heat formed by oxidation &c., and to cool down the rock as it is opened out.

About 700 men are employed at Dolcoath. They work in three shifts. The mine is almost everywhere damp, so that much mud adheres to their boots, clothes, and hands. This mud is carried about, particularly upon the boots, and is necessarily deposited upon the rungs of the ladders, from which it is conveyed to the hands of the next man who ascends or descends (see Plates I. and II.). Thus if the mud is contaminated with infective material it becomes almost impossible for the men to avoid infection. The clothes used in the mine are changed in a building on the surface provided with drying accommodation and lavatories, and known as the "dry." The men can thus come to and go from the mine dry and clean. The clothes become wet during work, partly from water and mud in the mine, and partly from perspiration caused by working in a warm and saturated atmosphere. In the warmer places the men work stripped to the waist, as in collieries.

Plate III. shows an underground meal, or "croust." The food, consisting of "pasties," is commonly carried in a small bag, which greatly contributes to cleanliness. The water is carried in a wooden keg (seen in the foreground), from which the men drink directly. The mine water is not drunk by the men. The kegs are certainly liable to contamination with mud unless great care be exercised, since the bung is constantly in contact with muddy hands and clothes, not to speak of mud from the ground and from the faces of miners. The pipes of men who smoke are also contaminated with mud.

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[1] Just after the paper was written the whole of the main roads had to be abandoned on account of a great fire due to spontaneous combustion.

In English mines there is, so far as we are aware, no privy accommodation underground, and Dolcoath has been no exception to this rule, although ample accommodation is provided on the surface. Owing to the thoughtless habits of a number of the men the ground has been polluted by faecal deposits at many parts. In the imperfect light it is impossible to avoid treading on these deposits, so that the ankylostome ova and the larvae which have developed from them in the warm and moist atmosphere, must be carried on the boots of the men to the roadways, ladders, skips, &c. Both in Dolcoath and in a neighbouring mine we have repeatedly found ova in the faecal deposits, together with very numerous larval worms apparently identical with those of *Ankylostoma*. Samples brought from various parts of Dolcoath were in every case infected with these ova or larval worms. The openings of disused levels are specially contaminated by excrement, but other parts are also made use of by the men. At intervals in the shaft there are large cisterns, from which the water is pumped by the shaft pumps to cisterns above, and thence to the surface. These are to a certain extent used by the men for receiving their evacuations; and this plan seems much less open to objection, as the faeces are pumped to the surface, where they can probably do no harm; and running water is said to prevent in any case the development of the ova.

Origin and Progress of the Outbreak in Dolcoath Mine.

At what time and by what means ankylostomiasis was first introduced into Cornwall it is now impossible to determine accurately; but it seems probable that the disease was brought, not by men from infected mines on the Continent, but directly from some tropical country. Cornish miners in charge of machine drills, or various departments of mining work, are now constantly going and coming from various parts of Asia, Africa, North and South America, and Australia; and the manufacture of mining machinery is a very important Cornish industry. As ankylostomiasis is endemic in many of the places from which these miners return, there is every likelihood that a good many of them will return infected, although possibly with so few ankylostomes that they are not anaemic. In examining blood-films from a number of men who had not worked in Dolcoath, or so far as we could ascertain been in any way exposed to ankylostomiasis infection in Cornwall, we found one sample of blood in which the eosinophile leucocytes were increased to 24 %. See Appendix, case LX. As shown below, this is one important

symptom of infection.   Although the miner had no definite symptoms of
illness we obtained a sample of his faeces, and found that ankylostome
ova were present, though in small numbers.   On enquiry we found that
he had returned from Mysore several months previously, where he had
doubtless been infected, though only slightly.   There have doubtless been
many such cases in recent years ; and from this example it may easily
be seen how the infection has reached Cornwall.

The outbreak at Dolcoath appears to have begun about eight years
ago.   Some of the affected men sought treatment in the West Cornwall
Miners' Hospital at Redruth, about three miles away ; and the following
record of cases admitted for anaemia since 1893 affords some idea of the
course of the outbreak.   Of the cases recorded in the table 61 % were of
miners directly from Dolcoath.   Only one death occurred.

TABLE II.

Year	Cases admitted
1893	1
1894	3
1895	9
1896	13
1897	29
1898	23
1899	12
1900	11
1901	7
1902 (till December) ...	8

The anaemia first began to attract attention at the mine about six
years ago.   The cases appear to have occurred almost entirely among
men working in and around the Engine Shaft, where a large number of
the men are employed.   Practically everyone in this part of the mine
seems to have been more or less affected, including the Manager and
nearly all the officials employed underground.   The position of this
shaft, with the temperatures observed in it, is shown on the section
(Figure 1).   It will be seen that it is an upcast, and considerably warmer
than the downcast shafts.

About the end of 1898, when the numerous cases of anaemia were
causing much trouble, the Manager, Mr R. Arthur Thomas, was led to
suspect that the occurrence of what the miners called "bunches" (a
skin affection which will be described below) was connected with
contamination of the mine by faeces.   He accordingly provided
additional privy accommodation on the surface, and requested the

men to avoid polluting the mine. He also ordered a large quantity of chloride of lime and permanganate of potash to be used at polluted spots in the mine and shafts. At the same time the ventilation was improved by various means; and finally, at the beginning of 1900, four centrifugal fans were placed on levels close to the Eastern Shaft at the places shown on the section, so that fresh air was blown through doors towards the Engine Shaft. By this means the temperature of the Engine Shaft and adjoining workings was apparently lowered distinctly, the improvement in ventilation being evident to Mr Martin when he re-visited the mine. The number of cases of anaemia has diminished greatly during the last two or three years, probably owing to these measures, although the possibility must also be borne in mind that some form of immunity to the effects produced by the presence of the worms in the intestine may have developed among the miners.

Fortunately there is surface employment for a large number of men at Dolcoath, and many of the men have been transferred to this or to work in the downcast shafts, as soon as they began to be seriously anaemic. Others have gone into Hospital or been sent to a Convalescent Home for a time, while others have obtained employment elsewhere. In the great majority of these cases the tendency has been towards more or less complete recovery. When the opportunity for renewed infection is prevented the worms existing in the intestine evidently tend to die out, as they cannot multiply within the body; and the anaemia correspondingly diminishes. Some of the worms may, however, remain in the intestine for years, as will be shown later. Of the cases which we have seen only one was extremely grave and it improved rapidly after treatment with thymol. Some idea of the number of men at present affected will be afforded by the particulars of cases in the Appendix.

In spite of many enquiries we have been unable to obtain any evidence of spread of the disease above-ground: but there is little doubt that the mines in the neighbourhood are more or less infected. We have examined a number of the men working in mines at some distance, but except in men who had been previously working in Dolcoath, or lately returned from abroad, we could see, or hear of, no evidence of definite anaemia. This is the more remarkable as probably nearly all the men working in Dolcoath have been, or are, more or less infected. It must be borne in mind, however, that many mines are much shallower and cooler than Dolcoath, and that the men may, perhaps, be cleaner in their habits.

Symptoms observed.

With certain possible exceptions mentioned below, the most important symptoms which the infected miners exhibit depend on the anaemia which is, in many instances, produced. Pallor, particularly of the lips and conjunctivae, together with palpitations and dyspnoea on exertion are the three chief signs which ankylostomiasis produces in common with any other anaemia. Underground work in Cornish mines always involves a good deal of climbing up almost vertical ladders. This is a form of exertion which soon brings into prominence any tendency to dyspnoea, and provides the basis for a very useful test question. A good many of the milder cases complain of palpitations only, though in some the dyspnoea is recognised without any excessive cardiac action being noticed. It is interesting to notice that the degree of dyspnoea does not altogether vary with diminution in the percentage of haemoglobin. It is a little surprising that hard manual labour can in some instances be done without difficulty with a haemoglobin percentage of 36—38 of the normal (cases XXXIX. III. IV., Appendix), and several further examples will be found in the table of cases of regular pit work being done with less than 50 p.c.[1] On the other hand there were examples such as case I, where with 48 p.c. there was marked dyspnoea on walking on the flat. Dizziness and faintness on exertion were complained of only occasionally.

The dyspnoea and palpitations are undoubtedly due to the deficient percentage of haemoglobin and red corpuscles, but do not appear to be caused simply by the deficient oxygen-carrying power of the blood. Where the symptoms are due solely to deficient oxygen-carrying power (as in carbonic oxide poisoning[2]), dizziness, loss of power, and fainting are by far the most prominent symptoms. Palpitations are also present, but dyspnoea is hardly noticed. In anaemia, on the other hand, dyspnoea is very prominent, and is probably due to rise in the carbonic acid tension of the blood. It is well known that the ease with which blood gives off its carbonic acid depends upon the presence of the red corpuscles. Any deficiency in the latter will thus diminish the difference in carbonic acid percentage between venous and arterial blood, and thus tend to produce carbonic acid dyspnoea.

[1] B. K. Ashford (in Cabot's *Clin. Exam. of Blood*, 4th ed., 1901, p. 430) has also drawn attention to this peculiarity.

[2] Haldane, *Journ. of Physiol.*, Vol. XVIII., pp. 201, 430.

Oedema has not been a marked feature of the Cornish cases : indeed only one of our cases has a history of obvious general oedema ; and swollen feet were curiously absent from the general histories. No traces have been found of petechiae, retinal haemorrhages, &c.

Gastrointestinal disturbances have been present in most of the cases of definite anaemia. The loss of appetite, dyspepsia, and occasional vomiting, which were frequent though never very prominent symptoms, may reasonably be ascribed to the anaemia. On the other hand many cases had nothing except a certain amount of epigastric pain, in some instances amounting to nothing more than epigastric uneasiness. This was not associated particularly with the taking of food. In one case a moderate degree of tenderness was elicited by the pressure over the region of the duodenum. Irregularity of the bowels was present in most cases, alternating diarrhoea and constipation being perhaps the commonest condition. Some complained of either diarrhoea or constipation : others seemed to be normal in this respect. In no case could any history of melaena be obtained, and in none of the samples of faeces examined was the colour suggestive of bleeding into the alimentary canal. It is probable that these symptoms as well as the pains are, in part at least, due to the presence of the intestinal parasite, though any of them might be due to the anaemia alone. The most anaemic case which came under our notice (XI.) was singularly free from any disturbances of the intestinal tract.

In one case (I.) absence of knee-jerks with an indefinite dulling of tactile sensibility over the legs was observed. Systematic examinations into these nervous affections were not made. Fever seems to be absent both at the onset and later, except in cases of marked anaemia which may show a low irregular pyrexia.

The miners complained a great deal of certain skin affections, and among themselves associate these eruptions very definitely and emphatically with the anaemia. It has already been stated that the worse degrees of " Dolcoath anaemia " are popularly associated with the " New Sump," or Engine Shaft. Our enquiry has shown that this association, though less absolute than the miners would have one believe, is actually borne out by facts. In the same way the skin affections are described as " New Sump bunches " or " botches." They appear very rapidly, are extremely irritable, and sometimes do and sometimes do not come to a head. They are very commonly attributed to sitting upon or leaning against the rock, and more especially the timber, underground. There are a number of men who only go down

the New Sump shaft on Saturdays, when they are engaged in repairing the pumping machinery : it seems to be almost a cause of surprise with them if by the same evening, or at latest the next morning, some " bunches" have not made their appearance. They are specially liable to appear on the buttocks, knees, and forearms. In several cases the "bunches" have been described as definitely preceding by a short interval the onset of anaemic symptoms. With the decline in the number of cases of anaemia which has taken place in the last three years, these " bunches" have also become much less frequent. It has therefore not been possible to see a great number of cases. From those we have been able to examine it would appear that the cutaneous affections may be described under three headings :—

(1) *Furuncles* of varying size, some being no more than pin-head pyodermias, others containing as much as a drachm of pus. Even the smaller ones have a great deal of hard thickening round the actual purulent focus. In some cases, at any rate, their origin from infections of hair follicles seemed clear, the follicle being filled with a plug of inspissated pus leading to a larger quantity of pus situated beneath. In many instances these furuncles gradually disappear without any pus making its appearance on the surface. To the naked eye the pus presents no unusual appearance.

In view of the recently published evidence[1] that *Ankylostoma* larvae may penetrate the hair follicles, the examination of the contents of these furuncles was of considerable interest. Nothing in the way of larvae or any other phase in the life-history of *Ankylostoma* could be identified in the pus. If these furuncles are specific it is probable that histological examination of the pus would reveal an excess of eosinophile cells similar to that found in the blood, while if they are due to ordinary pyogenic infection of the skin of persons infected with *Ankylostoma* and showing an eosinophilia in the blood, it is to be expected that the pus would consist of the ordinary polymorphonuclear neutrophile cells. If, again, the infection is primarily specific and becomes secondarily infected, an excess of eosinophiles in the pus might be either mixed with or obliterated by an excess of neutrophile cells. On microscopical examination of films of pus from these furuncles abundant groups of *Staphylococci* were found. Nearly all the cells were the ordinary neutrophile leucocytes in various stages of degeneration. An occasional eosinophile cell could, on prolonged examination, be detected

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[1] Looss (1901), *Centralbl. f. Bakteriol.*, Bd. xxix. pp. 735—739.

Fig. 1.   Posterior end of adult male
' Ankylostoma, ⨯ 40.
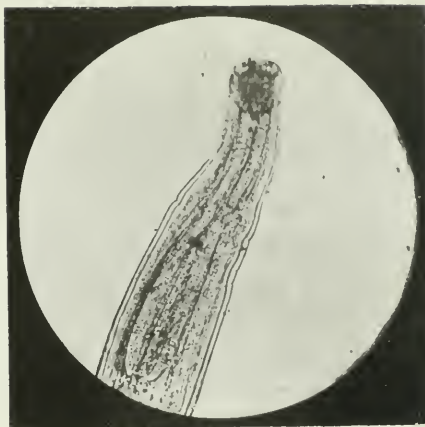
Fig. 2.   Head of adult Ankylostoma, ⨯ 30.
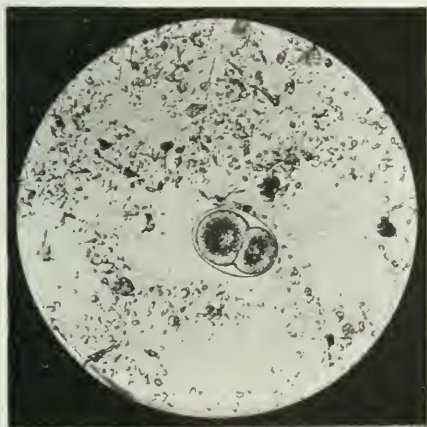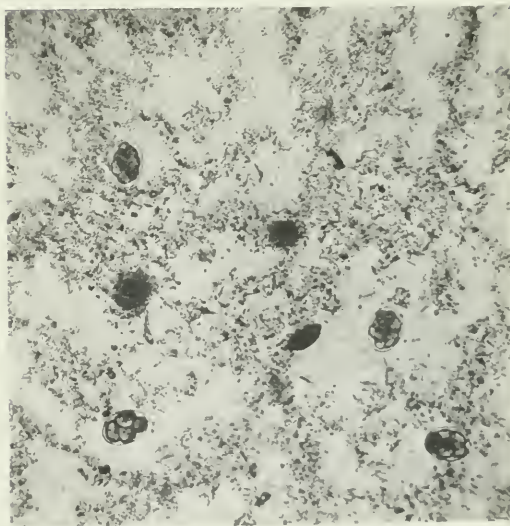

Fig. 3.   Two-cell stage of developing
ovum, ⨯ 200.

Fig. 4.   Ova at different stages.   Near centre
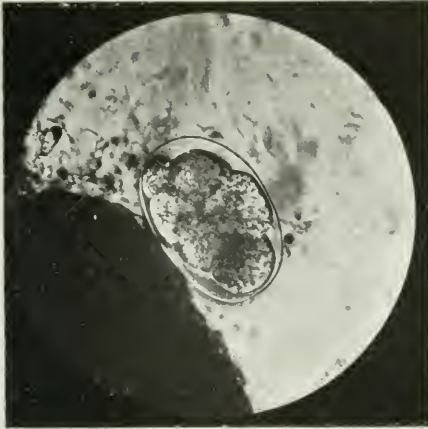an ovum of *Trichocephalus dispar*, ⨯ 50.
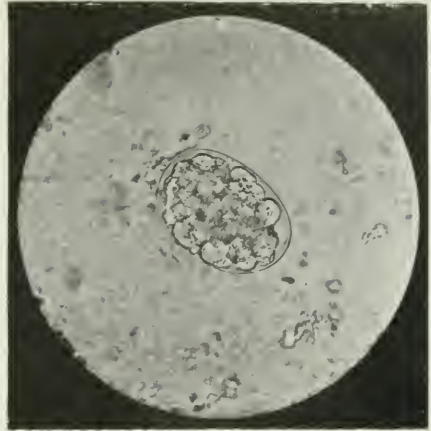
Fig. 5.   Eight-cell stage of ovum, × 400.
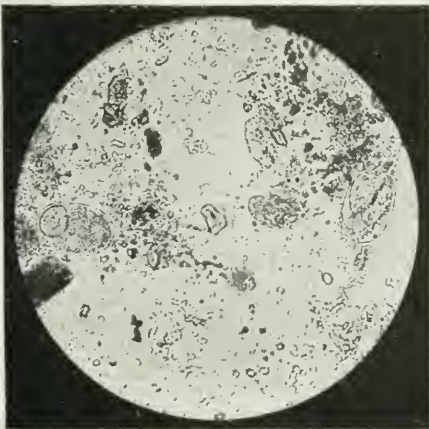

Fig. 6.   Morula stage of ovum, × 400.

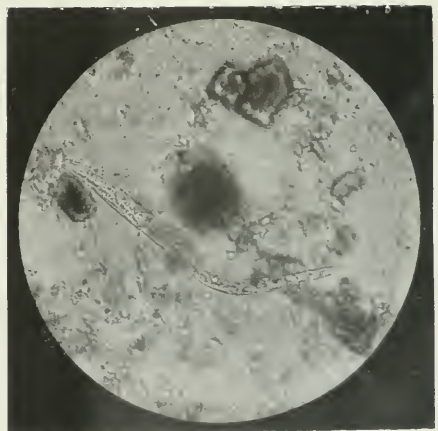
Fig. 7.   Ova showing larval worm coiled
up inside, × 160.

Fig. 8.   Recently hatched larval worm

in all the cases examined, but in no case under similar circumstances did we fail to find a certain number of red blood corpuscles. The possibility also that some of the smaller boils arose from scratched wheals of urticaria (see below) must be considered. In no instance was anything found which even approached eosinophile pus. On the other hand, the presence of the cocci renders possible the explanation that a specific eosinophile reaction on the part of the leucocytes towards an ankylostoma infection had been overwhelmed by a neutrophile reaction induced by a secondary infection with pyogenic organisms. It should perhaps be mentioned that among those cases from which some pus was obtained was the lad (case XII.) whose blood showed an extreme instance of eosinophilia.

(2) *Urticaria.* This seems to be common, though hardly so frequent as the furuncular affection. It is difficult to ascertain exactly what the relative frequency is, for the wheals are often infected by scratching, and so fail to entirely disappear in a few days. There is, however, no doubt that true urticaria often occurs, and the extremely irritable variety of "bunch" which spreads rapidly about the body and may disappear again in a few hours may be taken as being urticarial in nature. Urticaria is more commonly due to general dissemination of toxic substances in the body than to local external applications, and it seems not unlikely that it is in the present instance due to the presence of *Ankylostoma* in the gut. There is no doubt that urticaria is very much more frequent among infected miners than it is among the general population. Unfortunately there seem to be few if any underground workers at Dolcoath who do not harbour the worm, so that the influence of the hot, moist atmosphere and the profuse sweating cannot be excluded. At St Agnes the miners do not seem to be generally infected (see Appendix), and urticaria is not more frequent than among a normal population. Here, however, the mine is shallow and relatively cool, so that the two cases are not parallel. The majority of cases at Dolcoath, since leaving the shaft or pit and working on the surface, have never had any "bunches" at all, either furuncular or urticarial, though they were having constant attacks while underground. But in the case of James W. (XXXVIII.), who has kept the house for the past 20 months, six miles from Dolcoath, attacks of urticaria have been frequent since giving up work ; so long as he worked underground he suffered almost constantly from small boils which he has never had since he left the mine. Such a case as this renders it not improbable that the urticaria is comparable to that which is sometimes associated with hydatids.

In two cases differential counts of the leucocytes have been made, (*a*) in blood and serum squeezed from the fresh, uninflamed wheals of an urticaria in the leg, (*β*) in blood from the finger. The results are as follows :

		Lympho-cytes	Inter-mediate	Large hyaline	Neutro-phile	Eosino-phile	Mast-cells
P. W. J.	urticaria	28·7	6·6	1·0	36·7	27·0	0
	finger	12·3	2·0	3·3	51·7	30·0	0·7
W. J. J.	urticaria	40·0	7·3	4·3	35·0	13·3	0
	finger	29·7	3·3	2·0	55·3	9·3	0·3

These figures yield evidence of an uncertain character. The marked excess of lymphocytes in the wheals suggests that it might be more proper to compare the eosinophiles to the neutrophiles rather than to the total leucocytes. If this is done the proportion of eosinophiles in the finger blood to those in the wheal becomes 100 : 126 and 100 : 237 in the two cases respectively.

(3) *General pruritus.* A few of the cases find a general pruritus without any objective signs, and especially marked over the trunk, a very troublesome complaint. As usual, it is most marked at night, though it is interesting to note that in one instance this feature still held although the individual was working all night and slept during the day. In the same case, the expulsion of more than 200 worms by thymol resulted in a complete cessation of the irritation for about a week ; it then returned, though in less degree, and at the same time it was found that ova were still present in the faeces in some numbers. This pruritus is not a common symptom (if it be really a symptom of ankylostoma infection). It occurs equally in the old and young; thus cases I. aged 51 and VI. aged 20 presented the worst degrees, and neither was exposed to fresh infection.

Attention may finally be called to the fact that in some cases, among which is the most anaemic of all (XI.), there is no history of any skin affection whatever to be obtained.

The definite diagnosis of ankylostomiasis depends, of course, on the recognition of the ova in the faeces. As a rule there is no difficulty in finding the ova. All that is necessary is to mix a small fragment of the faeces with a drop of water spread out on a slide, and search with a low power. Plates IV. and V., which are from micro-photographs kindly made for us by Dr C. A. Coventon at the Pathological Laboratory, Oxford, show the appearances presented by the ova at different stages of development, and by the larval and adult worms. Figures 3 to 6 show the ova as seen in fresh faeces.

Changes in the Volume of the Blood.

The essential symptoms of ankylostomiasis are those of what is commonly known as anaemia; and for practical purposes the word "anaemia" has now lost its etymological significance, and denotes simply any condition in which the percentage of haemoglobin in the blood is abnormally low. Indeed, no case in which there was definite evidence of anaemia in the etymological sense has ever been recorded. It is evident that anaemia may be produced, either by a diminution in the total haemoglobin in the vascular system, without a corresponding diminution in the total volume of the blood, or by an increase in the total volume of the blood, without a corresponding increase in the haemoglobin. It has recently been shown by Lorrain Smith[1] that pernicious anaemia and anaemia from haemorrhage are of the former, and chlorosis of the latter type. It was evidently of importance to ascertain to what type the anaemia of ankylostomiasis belongs; and we have therefore determined the total volume of the blood in several cases.

The method used was that of Haldane and Lorrain Smith[2]. The principle of this method is as follows: (1) The percentage of haemoglobin is determined colorimetrically, and from the result the percentage capacity (by volume) of the blood for absorbing oxygen or CO in combination with the haemoglobin is calculated. (2) A known volume of CO is administered to the patient. (3) The percentage to which his haemoglobin is saturated with CO is determined colorimetrically immediately after the absorption of the gas. It is evident that from the percentage saturation actually observed, as compared with the volume of CO absorbed, it is possible to calculate the CO (or O_2) capacity of the whole of the blood—*i.e.* the volume of CO or O_2 required to saturate the whole of the haemoglobin in the blood. As, however, the percentage O_2 or CO capacity of the blood is also known, the total volume of the blood can easily be calculated. Thus if the corrected volume of CO absorbed was 75 c.c., the percentage saturation of the haemoglobin with CO 15, and the percentage O_2 capacity of the blood 18·5, the O_2 or CO capacity of the whole blood would be $75 \times \dfrac{100}{15} = 500$ c.c., and the total volume of the blood $500 \times \dfrac{100}{18 \cdot 5} = 2700$ c.c. If the body-weight (without

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[1] *Trans. of the Pathological Society*, Vol. li. p. 311.

[2] *Journ. of Physiology*, Vol. xxv. p. 33, 1900.

clothes) were 60 kilos the oxygen capacity in c.c. would be ·83, and the blood-volume 4·5 c.c. per 100 grammes of body-weight.

The haemoglobin was determined by an accurately graduated and standardised Gowers-Haldane haemoglobinometer[1], the reading of 100°/₀ by which corresponds to an oxygen capacity of 18·5 c.c. per 100 c.c. of blood. The carbonic oxide used was analysed after each experiment; and the volume administered was measured from a burette instead of the measuring cylinder originally described. The standard blood solution employed in the titrations was made from fresh human blood, and was found to give exactly the same tint at corresponding dilutions as the patients' blood.

Our first two experiments were made under the belief that the anaemia of ankylostomiasis is probably due simply to loss of blood through intestinal haemorrhage caused by the presence of the worms, and to a subsequent more rapid reproduction of the plasma than of the haemoglobin[2]. If this were the case the total haemoglobin in the vascular system would be greatly diminished, and consequently only a small volume of CO could be administered without saturating the haemoglobin to such an extent as to produce symptoms. We therefore only gave small amounts of CO. Somewhat to our astonishment the saturation of the haemoglobin was in each of the two cases so low that very accurate titrations could not be made. It was evident that the blood-volume was greatly increased, and we therefore gave more CO in the subsequent experiments. The following table gives the results.

Case-number in Appendix	c.c. of CO absorbed at 0° and 760 mm.	% saturation of haemoglobin with CO	Oxygen capacity of total blood	% of haemoglobin normal = 100 %[3]	Percentage oxygen capacity of blood	Total volume of blood in c.c.	Body-weight in kilos	c.c. of blood per 100 grms. body-weight	c.c. of oxygen capacity per 100 grms. of body-weight
I	41	8·2	500	44	8·1	6170	72	8·6	·70
VI	41	8·5	480	47	8·7	5520	60	9·2	·80
VI	72	15·9 16·5 } 16·2	445	41	7·6	5860	60	9·8	·74
X	72	19·4 19·7 } 19·5	375	49	9·1	4070	53	7·7	·70
Average	—	—	—	45	8·4	—	—	8·8	·735
Average for 12 healthy men	—	—	—	100	18·5	—	—	4·5	·83

[1] *Journ. of Physiology*, Vol. xxvi. p. 497, 1901.

[2] See *Cohnheim's Allgemeine Pathologie*, Vol. i. Chap. vii. (2nd edition).

[3] These values differ from those given in the Appendix: they were determined on other days.

It will be seen by reference to the Appendix that cases I. and VI. were of old standing in men of 51 and 20, while case X. was very recent, in a lad of 17. In each case the volume of the blood was increased far beyond the normal limits recorded by Haldane and Lorrain Smith in the paper just quoted. The average increase amounted to 94 %. The total oxygen capacity (which is a measure of the total haemoglobin in the blood) was only slightly diminished (by 11 %). It is thus evident that the condition of the men examined was essentially one of hydraemic plethora. If the blood had been concentrated to its normal volume the haemoglobin percentage would have risen from 45 % to 89 % of the normal. The slight remaining deficiency may perhaps be set down to the haemorrhages which, from post-mortem records, seem undoubtedly to occur in cases of ankylostomiasis.

In cases I. VI. X. the colour indices of the red corpuscles were ·74, ·79, and ·58 (see Appendix). It follows from this that the total number of red corpuscles in the blood was in each case a good deal above the normal—in case No. VI. by as much as 42 %.

For comparison's sake we add the following table, showing Lorrain Smith's average results for anaemias of other type.

	% of haemo-globin (nor-mal = 100 %)	Red cor-puscles in millions per cubic mm.	c.c. of blood per 100 grms. of body-weight	c.c. of oxy-gen capacity per 100 grms. of body-weight
Six cases of anaemia from haemorrhage	33	3·067	6·5	·39
Seven cases of pernicious anaemia	26·5	·940	8·6	·40
Twenty-one cases of chlorosis	39·9	3·222	10·8	·79
Four cases of chlorosis after iron treatment	74·0	5·399	6·2	·84
Six healthy young women	93·0	4·952	5·3	·92

It will be seen at once on comparing this with the previous table that the anaemia of ankylostomiasis differs greatly from anaemia of haemorrhage or pernicious anaemia, but closely resembles the anaemia of chlorosis. It is true that the volume of the blood is usually increased, and that the colour index of the red corpuscles is low in anaemia from haemorrhage, but the difference in the total oxygen capacity of the blood sharply differentiates the two conditions. In the cure of ankylo-stomiasis anaemia by vermifuge remedies the same rapid concentration of the blood doubtless occurs as Lorrain Smith has demonstrated in the cure of chlorosis.

In the absence of further experimental data there is little use in discussing the means by which the anaemia of ankylostomiasis is brought about. The most commonly accepted explanation, that the anaemia is directly due to loss of blood from the punctures of the worms, is however, evidently incorrect.

Changes in the Constituents of the Blood.

The changes in the constituents of the blood have been examined in a number of cases. It has been impracticable to enumerate the cells in all instances, but in each case the haemoglobin has been estimated and stained films examined. It is from these examinations perhaps that the most reliable and useful information is to be gained. The haemoglobin was estimated by Haldane's modification of Gowers' haemoglobinometer, an accurately graduated and standardised instrument being employed. Where gas was not available an original Gowers standard of known error was used. The cells were counted with the Thoma apparatus, using Toisson's diluting fluid and a Zappert counting-chamber, and counting 100 small squares for the red cells and 9 sq. mm. for the leucocytes. In the differential leucocyte count 500 leucocytes were counted except in one or two instances. Though other stains were occasionally used, as a routine the films were stained with different varieties of Jenner's solution[1].

It is important in considering the results of the blood examinations to appreciate the errors which may be present, arising either from the method of experiment used or from the circumstances under which the different examinations have been made. The error of the haemoglobinometer has been shown (Haldane, *loc. cit.*) to be very small (less than 1 p.c.). In counting the red cells by the method here used the error is commonly said to be about 3 per cent., but this is probably far too optimistic a view to take of the accuracy of the method in general practice. That there is a considerable error in counting the leucocytes by the Thoma method is generally admitted, but to what dimensions this error may rise is not known. The differential count would appear at first sight to be liable to but a very small error; but successive counts of 500 or 1000 cells in the same film show that the successive results may vary a good deal in cases where there is no question of any error of judgment in assigning each cell to its appropriate class.

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[1] We have to thank Dr Gustav Mann for a supply of a particularly admirable sample of eosinate of methylene blue in methyl alcohol, which gave excellent results.

Thus in two cases the figures for the neutrophiles in each of four successive thousands of white cells were;

59·2	55·5	55·2	53·6 per cent.
35·8	32·7	38·7	34·8 „ „

In view of a tendency to lay too much stress on small differences in blood-counts it seems well to call attention to the fact that an error of method is present and that it is not altogether negligible in magnitude.

A further source of error in comparing the different cases which we have collected arises from the fact that it has been found utterly impracticable in dealing with an active body of working men to ensure that all the estimations should be done at the same time of day and in the same relation to the taking of food. As a matter of fact however the present estimations were almost all made at a time after the last considerable meal, when digestion leucocytosis has become practically negligible, and which was in a large number of instances roughly the same. The fluctuations which take place from week to week in normal persons who show no signs of illness must also be considered in comparing the results of single examinations in a series of individuals[1]. The possibility of the presence of other diseases which might influence the condition of the blood was carefully considered; the few cases where such was found are mentioned under the appropriate heading. We might however state definitely here that in none of our cases did we find any history or indication of asthma, skin affections, etc. which might account for the increase in the eosinophile leucocytes.

The classification of the different varieties of leucocytes is not quite the same as that commonly adopted. The term "lymphocyte" is confined to small cells with very little cytoplasm and a deeply staining nucleus. "Large hyaline" includes the well-known cell of large size with a (usually) indented nucleus which does not stain deeply, and fairly abundant cytoplasm containing abundant basophile granulations. Between these two well-marked classes are a considerable number of cells which in size and other characters lie in an intermediate position. The relative number of these "intermediate" cells varies a good deal in different films, and the cells which are classed together under this heading are an exceedingly mixed collection. So little is known of these non-granular leucocytes that further subdivisions would in the present instance serve no useful purpose. To obtain figures

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[1] See for example the differential counts in cases II. and XL. and contrast those in case XII.

comparable to other differential counts the "lymphocytes" and the "intermediates" should be taken together to represent the lymphocytes of the ordinary classification.

Literature. Without attempting an exhaustive survey of the literature it seems proper to give some account of the results which have been obtained by others in examining the blood in cases of ankylostomiasis. With really only one exception the published observations are rather fragmentary. A few cases are described, chiefly with reference to the increase in eosinophile leucocytes, by Zappert and others[1]. The figures of Leonard Rogers[2] have been republished more than once and constitute perhaps the best known work on the subject. They deal with the total red cells, haemoglobin, total leucocytes and specific gravity of about a dozen cases among the natives in Assam. His average figures are: Hb 15·2 p.c., red cells 2,145,000, leucocytes 5338, sp. gr. 1·034, and colour index 0·35. The haemoglobin estimations were made with the original instrument of Gowers, and are on that account to be regarded with great suspicion in the absence of definite standardisation of the apparatus, particularly for low percentages. There are indeed data which lead one to suppose that Rogers' standard was too low: he states[3] that the average haemoglobin percentage for healthy Europeans resident in Assam is about 80 in the healthier season, and 71 p.c. in the rainy season, the average for healthy Assamese being 62. On the contrary, other workers, in investigations conducted with a considerable degree of thoroughness, appear to have established the fact that the haemoglobin and red cells of healthy persons, both

[1] J. Zappert, *Wiener klin. Wochenschr.* 1892, p. 347, and *Zeitschr. f. klin. Med.* XXIII. 1893, p. 257; Bucklers, *Münchener med. Wochenschr.* XLI. 1894, p. 22; Müller and Rieder, *Deutsches Archiv f. klin. Med.* XLVIII. 1891, p. 114; Leichtenstern, *Wiener klin. Rundschau,* 1898, pp. 413 and 429. Some other references are given by B. K. Ashford (*loc. cit.*), and in Scheube, *Krankheiten der warmen Länder,* 1900.

[2] The original table (*Report on Kala-Azar,* Shillong, 1897, p. 95) is as follows:—
Hb % 15·2; Reds 1,145,000; Whites 5,338; Whites : Reds 1:524; sp. gr. 1·034; Hb value 0·31. It is at once obvious that this is not correct, for the colour index calculated from the Hb and reds as given is 0·65. From the ratio whites to reds and from calculating the reds from the colour index and the Hb it appears that the correct number for the reds is the figure (2,145,000) given by Rogers in the *Journ. of Pathol. and Bacteriol.* v. 1898, p. 399. The original table appears in the *Brit. Med. Journal,* 1900, II. p. 544, and is reproduced by R. C. Cabot, *Clinical Examination of the Blood,* ed. 4, 1901, p. 428, though the data for detecting the error accompany it. If the different columns in the table apply to the same series of cases, the colour index and proportion of whites to reds are also wrongly stated; the former (0·31) gives with 15·2 per cent. Hb about 2·45, the latter about 2·8 million reds.

[3] *Journ. of Pathol. and Bacteriol.* v. 1898, p. 399.

Europeans and natives, resident in the tropics are not reduced[1]. It is therefore not improbable that the haemoglobin percentages given by Rogers are all some 20–30 per cent. at least lower than they should be, and, if that is so, it follows that the very low colour index, on which the author lays so much stress, is also too low. The results with the original Gowers instrument in cases of extreme anaemia are particularly unreliable, as the method of graduating the instrument was quite unsound, and tended to make the results much too low even when the standard picro-carmine tube was of the correct tint.

F. M. Sandwith[2] records the results of the examination of the blood in 173 cases in hospital in Cairo. The haemoglobin (estimated by the original Gowers method) varied from 10 to 54, with an average of 26 p.c. One of the most interesting points which this author brings out is that during the process of cure the number of leucocytes per cub. mm. increases[3] (average on admission 10,360 and on discharge 15,730 per cub. mm., the average gain in red corpuscles being 1,290,000, and in haemoglobin about 27 p.c. in the same cases).

The most careful and complete account which we have seen is one by B. K. Ashford[4] dealing with 19 cases at Puerto Rico. The blood-counts were done in duplicate and the Hb estimations (with a Fleischl apparatus) in triplicate. His averages are: Hb 21, red cells 1,776,000; leucocytes 7000; colour index 0·6. In the differential count, the average percentage of eosinophiles is 10·3, six of the nineteen cases being under 5 p.c. Poikilocytosis was present in most cases, with abundant megalocytes and microcytes; polychromasia was only marked in four instances. Normoblasts were present in fourteen cases and in six were accompanied by megaloblasts; in no case however did the latter predominate. Two of his cases had a colour index of more than unity— 1·43 and 1·09. Good examples are given of the extremely rapid changes, both backwards and forwards, which may take place in a short

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[1] C. Eijkman (*Virchow's Archiv*, cxxvi. 1891, p. 113), using a Fleischl haemoglobino-meter and a Thoma-Zeiss counting apparatus, found about 5·2 million reds and 96·5 to 100 per cent. Hb. Similar results (reds and sp. gr.) were obtained by Max Glogner (*ibid.* p. 109).

[2] *Lancet*, 1894, i. p. 1365. Some determinations of the volume of the red cells by Kaufmann are given here (15—43, av. 25 p.c.), but the numbers of cells in the same cases are not recorded.

[3] In the single case (XI.) which we have had an opportunity of observing, the leucocytes *fell* 40 per cent. while the Hb increased 20 per cent.

[4] *New York Med. Journ.* lxxi. 1900, p. 552: a rather more convenient account has been contributed by the author to R. C. Cabot, *op. cit.* p. 429.

time : thus one case in 20 days gained three p.c. Hb and nearly two million red cells, reducing the colour index from 1·43 to 0·43. Ashford states that there is probably no true leucocytosis in ankylostomiasis[1], and endeavours to account for the leucocytoses which have been observed as being due to intercurrent diseases. Among his cases are three in which there is some increase in the white cells—11,000, 12,700, and 18,000. The second of these has 6 p.c. of eosinophile cells and no intercurrent disease ; the first has 31 p.c. and an abscess of liver, while the third has 40 p.c. eosinophiles and is "believed" to have had pneumonia at the time of examination. Among the other 16 cases in no instance does the percentage of eosinophiles rise above 17. So far from demonstrating by these figures that there is no leucocytosis, the author would seem to have advanced evidence of some weight in favour of the opposite view. It is going out of the way to suggest that pneumonia may cause a leucocytosis of 18,000 of which 7,200 are eosinophiles. Such a condition is contrary not only to all observations and theory on both pneumonia and conditions which favour eosinophilia, but is the reverse of what has been actually observed in ankylostomiasis. Leichtenstern has put on record[2] the observation that the incidence of a croupous pneumonia reduced the percentage of eosinophiles in the blood of a patient suffering from ankylostomiasis from 72 to 6–7, recovery from the pneumonia raising the number again to 54 p.c. That the percentage of eosinophiles was as much as 40 in Ashford's case is to be rather taken as evidence that the belief that the patient had pneumonia was without very much foundation. In our five cases (X. XII. XIII. XXIX. XXXII.) with a leucocytosis of more than 15,000 special enquiries were made for any other malady which might induce such a result : none were found.

There is a widespread idea that the changes in the blood produced by ankylostomiasis are either sometimes or frequently indistinguishable from those associated with progressive idiopathic pernicious anaemia[3].

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[1] If, as is perhaps best, we confine the term ankylostomiasis to a condition of anaemia produced by *Ankylostoma*, there is probably more truth in this statement than if we include all cases of infection by *Ankylostoma*. J. B. Greene (*New York Med. Journ.* LXXV. 1902, p. 460) records a leucocytosis of 45,000.

[2] In P. Ehrlich and A. Lazarus, *Die Anaemie*, Part I. p. 113. It is hardly necessary to acknowledge the debt which we owe to the brilliant chapter on specific leucocytoses in this book when any question, such as the present one, requires discussion.

[3] R. C. Cabot, article in Hektoen and Riesman's *Pathology*, I. 1901, p. 458; J. Ewing, *Clinical Pathology of the Blood*, 1901, p. 420. On the other hand J. C. da

Such modern examinations as have been made (and they are few in number) hardly bear out this view, though of course the difficulty of the definition of what constitutes "pernicious" anaemia introduces a complication. Poikilocytosis, polychromasia, normoblasts, and megaloblasts have been described : on the other hand the colour index is nearly always very low, and the megaloblasts less numerous than the normoblasts. No investigations into the condition of the marrow appear to have been made. Ashford mentions a single example with a colour index of 1·4, and this seems to be the case nearest a "pernicious" type, except that there were more normoblasts than megaloblasts. With one somewhat doubtful exception, our own cases show nothing more than may be met with in severe chlorotic anaemias, though the total blood volume is not unlike that of pernicious anaemia.

Taking a somewhat elastic view of what constitutes pernicious anaemia, it cannot be said that it is other than an extreme rarity in ankylostomiasis.

A detailed account of the changes which we have found in a series of cases is given in the Appendix. The essential alterations consist in an anaemia of severe chlorotic type with a large increase in the total volume of the blood, a varying increase in the leucocytes, and a marked relative and absolute increase in the eosinophile cells.

The haemoglobin is diminished in very varying degrees in different cases. The extremes which we have observed in infected persons are 17 (case XI.) and 104 per cent. (case XXXVII.). The reduction in the number of red cells per cubic millimetre shows equally wide variations (1·5 to 5·4 millions), but is proportionately less than the diminution in the Hb percentage. The colour index is thus low. In a series of cases of such varying severity it is not possible to arrive at satisfactory average figures, but in those cases—16 in number—where full blood examinations were made and in which the Hb falls below 60 per cent. the figures are as follows. The maximum and minimum of red cells occur in the two cases giving the maximum and minimum of haemoglobin.

Costa, *Clinical Hematology*, 1902, p. 357, points out that it differs from *Bothriocephalus* anaemia in not simulating idiopathic pernicious anaemia, and Ewing, *op. cit.* p. 177, agrees that the similarity has never been properly demonstrated. Among other authors, H. Sahli (*Deutsches Arch. f. klin. Med.* xxxii. 1883, p. 421) and J. P. Maxwell (*Journ. of Tropical Medicine*, iv. 1901, p. 317) definitely state that, in cases which had reached a grave degree of anaemia, the red cells did not show the poikilocytosis, etc., of pernicious anaemia.

	Max.	Min.	Average
Haemoglobin per cent.	58	17	43·1
Red cells {per cent.	81	30·5	64
{millions per mm.³	4·072	1·533	3·188
Colour index	0·71	0·56	0·67
Leucocytes per mm.³	44,000	3,800	13,300

The actual highest and lowest colour indices occurring among these cases are 0·81 and 0·53. The lower indices appear to be associated with the lower percentages of haemoglobin; thus the average index for those cases (five) with from 30–39 per cent. Hb is 0·64, from 40–49 per cent. (six) is 0·68, and from 50–59 per cent. (four) is 0·75. Thus the more profound the degree of anaemia the less does it tend to show the high colour index characteristic of the pernicious type. In four instances (XIV. XVI. XXII. XXIX.) the colour index was found to be definitely above unity (1·11 to 1·38). Beyond the fact that the Hb percentages were high (89–98) no other peculiarity could be found in these cases, either clinically or in the blood.

Microscopically the red cells in the majority of cases exhibited nothing more than a deficiency in haemoglobin. In the single really severe case which we found (XI.), where but 1·5 million red cells were counted, a more than usually prolonged and careful search was made for the so-called "degenerative" signs of pernicious anaemia: a small degree of poikilocytosis and a more extensive amount of Gabritschewsky's polychromasia (only however in normocytes) were found; five normoblasts, four with polychromatic cytoplasm, and two doubtful megaloblasts were seen: megalocytes were present in very small numbers. In one other case a single, and in another (XVIII.) two normoblasts were seen; the latter case also showed two examples of punctate basophilia which occurred in single cells in two (III. and XXXIX.) other cases. A small amount of polychromasia was present in many cases, but seldom to a degree much greater than is sometimes found in bloods which cannot be regarded as other than normal. Poikilocytosis was only found in one instance (XI.), and there not to any extent. In several cases (*e.g.* X. XXIV.) a good deal of variation in size was present (diam. 4·5—10·5 μ). Our cases are not so severe as those which have been studied by others, most of the men having advanced a good way towards recovery by the time they were examined. This may be the reason why we have not found more morphological changes in the red cells.

The total leucocytes per cubic millimetre varied from 3,800 to 56,000, the average of the 16 distinctly anaemic cases being 13,300. It may be well to state at once that the reaction of the leucocytes to

Ankylostoma infection is independent of the reaction of the red cells and plasma-volume: by the time that a condition of marked anaemia has been produced the leucocytic reaction seems to have generally to a large extent passed away. The condition of the white cells, in short, in the anaemic disease called ankylostomiasis does not represent in full their response to the presence of the worm in the intestine. The four highest counts are all in youths with but a short history of illness and are cases which may well be recent infections :—

Case	Age	Total leucocytes per mm.³	History of illness	Hb p.c.
XXXII	23	56,000	a few weeks	80
XII	17	44,000	6 months	50
X	17	24,400	2 ,,	40
XXIX	18	20,600	6 ,,	98

These high figures are not necessarily associated with severe symptoms of any kind: case X. was severely anaemic, but case XII. was mild, and the other two had hardly any symptoms at all. If with these cases we contrast the four which show the lowest counts of white cells we find that they are old-standing cases :—

Case	Age	Total leucocytes per mm.³	History of illness	Hb p.c.
II	43	3,800	4 years	35
XVI	21	6,200	4 ,,	99
XXXIII	18	6,700	2 ,,	38
III	32 circ.	6,800	4 ,,	38

In the differential count of the leucocytes the outstanding feature is the absolute and relative increase in the number of eosinophile cells[1]. For the 16 cases already referred to with a distinct reduction in the Hb percentage, the average percentage of eosinophiles is 23 (11·4 to 72·7) as compared with a normal percentage of about 2·5 and the average absolute number 3,000 per cubic mm. (676 to 32,000) as compared with a normal of about 225. Among the series of 46 cases which are recorded in Section I. of the Appendix, two showed only a high normal percentage (XXXVI. 3·6 per cent.; XVII. 3·7 per cent.; and 459 absolute), and four others (VII. IX. XL. and XLIII.) failed to reach 10 per cent. at the first examination[2]. The following table shows the

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[1] The eosinophiles, probably chiefly on account of their size, are destroyed more than the other varieties in making films; hence the figures are a trifle too low.

[2] In case XL. a second count gave 16 p.c.: on the other hand case II. on a second examination only gave 9 p.c.

relation to the total number of leucocytes and to the percentage of haemoglobin :—

No. of cases	Total leucocytes per mm.^{3}	Average p.c. of eosinophiles		Average p.c. of haemoglobin	
5	> 14,000		47·7		63·0
4	13,000 to 14,000	23·2		71·5	
5	12,000 to 13,000	17·5	19·1	57·4	62·4
2	11,000 to 12,000	14·6		62·0	
4	10,000 to 11,000	14·2		69·5	
2	9,000 to 10,000	11·3	14·7	66·0	65·4
4	8,000 to 9,000	16·8		61·0	
2	7,000 to 8,000	15·3		72·5	
4	6,000 to 7,000	14·45	15·2	68·7	65·0
1	< 6,000	17·8		35·0	

This table shows that the proportion of eosinophiles bears no precise relation to the degree of anaemia as indicated by the haemoglobin percentage, but that it corresponds in a general way to the degree of leucocytosis which is present[1]. From this it follows that the higher percentages of eosinophiles are found in those cases which have the higher absolute numbers per cubic millimetre—in short, that the condition is one of eosinophile leucocytosis. Thus, using the figures already given, we find that with a leucocyte count of 13,285 and a percentage of eosinophiles of 22·9, the total eosinophiles per cubic mm. number 3,053; this deducted from the total leucocytes leaves a number (10,232), which is not in great excess of a variable normal. The next table shows the absolute figures of the differential counts of 12 cases. The last column gives the total leucocytes per cubic mm. after deducting the absolute number of eosinophiles: the results indicate that the leucocytes other than eosinophiles take a variable share in the high leucocytosis. Thus in case XXIX. deduction of the eosinophiles reduces a leucocytosis of 20,600 to a normal 9,000. On the other hand, cases X. and XXXII. show that the neutrophiles and non-granular cells may be about doubled when the leucocytes are increased about three- and seven-fold. We would emphasize the fact that the subjects of these

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[1] As was the case in *e.g.* T. R. Brown's classical case of trichinosis (*Journ. Exper. Med.* III. 1898, p. 315).

Case	Total leucocytes	Lymphocytes	Intermediate	Large hyalines	Neutrophiles	Eosinophiles		Mast-cells	Total leucocytes less eosinophiles
						Absolute	Per cent.		
"Normal"	8,000	1,880		240	5,600	240	3	40	7,760
II	3,800	631	312	220	1,938	676	17·8	23	3,124
I	8,800	1,179	651	546	4,946	1,408	16·0	70	7,392
XVIII	10,700	1,873	696	303	4,548	3,231	30·1	0	7,469
XXIV	12,300	1,304	1,107	664	5,264	3,936	32·0	25	8,364
XI	12,960	1,840	311	467	8,139	2,048	15·8	155	10,912
VI	13,100	865	1,755	2,044	5,633	2,698	20·6	105	10,502
XV	13,500	1,242	1,080	1,080	7,020	2,970	22·0	108	10,530
XIII	16,200	2,722	583	486	9,979	2,365	14·6	65	13,835
XXIX	20,600	4,326	865	247	3,420	11,577	56·2	165	9,023
X	24,400	2,440	1,854	1,025	11,810	7,076	29·0	195	17,324
XII	44,000	2,772	220	308	8,580	31,988	72·7	132	12,012
XXXII	56,000	3,024		1,568	14,336	37,072	66·2	0	18,928

high leucocytoses presented no morbid condition other than *Ankylostoma* infection which could account for the state of the blood[1].

With regard to the proportions and absolute numbers of the other varieties of leucocytes present little can be said. The figures indicate wide variations and the changes are irregular. For the 16 anaemic cases the average is:

Lymphocytes	14·4	per cent.	= 1,915	absolute
Intermediates	7·4	,,	= 984	,,
Large hyalines	5·9	,,	= 785	,,
Neutrophiles	48·7	,,	= 6,477	,,
Eosinophiles	23·0	,,	= 3,059	,,
Mast-cells	0·6	,,	= 80	,,

In view, however, of the independence of the anaemic and leucocytic reactions it would be more proper to study the question in those cases which show a marked leucocytosis. In the next table the total eosinophiles are deducted and omitted and the other varieties are given as percentages of the total leucocytes left after deduction of the eosinophiles. Only the five highest leucocytoses are thus dealt with.

Case	Total leucocytes	Total leucocytes less total eosinophiles	Lympho-cytes	Percentages of leucocytes other than eosinophiles formed by			
				Inter-mediate	Large hyaline	Neutro-philes	Mast-cells
XIII	16,200	13,835	19·7	4·2	3·5	72·1	0·4
XXIX	20,600	9,023	47·9	9·6	2·7	37·9	1·8
X	24,400	17,324	14·0	10·7	5·9	68·2	1·1
XII	44,000	12,012	23·1	1·8	2·6	71·4	1·1
XXXII	56,000	18,928	15·9		8·3	75·7	0

With one marked exception these figures show an almost normal condition. Case XXIX. has a large excess of lymphocytes, and on looking through the cases in the Appendix other examples of a similar condition will be found. The large hyalines in the same way are somewhat increased in a good many cases. In view of the possible

[1] Case XIII. had four fair-sized furuncles which were beginning to heal; XXIX. and XII. had remains of one and two small pyodermias respectively; while X. and XXXII. were free from skin troubles. None of them had urticaria.

association of mast-cells and eosinophiles the figures for the former are of interest: the average is distinctly higher than that which is usually regarded as normal[1], and in one case (LVI.) the high figure of 2·5 per cent. is reached. The higher percentage of mast-cells is not however associated with the higher percentage of eosinophiles, and in 6 cases (including XXXII.) out of 58 examples of infected persons none were seen. On the other hand none were recorded in the short counts of 4 out of 11 non-infected Cornish miners. It may therefore be concluded that *Ankylostoma* produces some increase in the mast-cells which is proportionately much less than, and is not parallel with, the increase in eosinophiles.

Of the six cases where the eosinophiles fall below 10 per cent. three (VII. IX. XVII.) show a diminution of neutrophiles with an increase in the lymphocytes, while the other three (XXXVI. XL. XLIII.) may be called normal counts. None of these cases were at the time of examination suffering from symptoms of anaemia in any but the slightest degree, though the haemoglobin was in two instances under 60 per cent., and there is no reason for thinking that any of them were recent infections.

The discovery of a few neutrophile myelocytes in one severe case (XI.) is of interest in view of their frequent association with pernicious anaemia. They have been previously recorded in ankylostomiasis[2].

No abnormal histological changes were found in the leucocytes. The eosinophile cells were large (average about 14μ) and well-formed, with abundance of granules; they seemed to be always rather larger than the neutrophiles. The nucleus was of the shape which is so characteristic of normal eosinophile cells, *i.e.* formed of two oval approximately equal parts, joined by a fine filament, the latter lying generally in fixed films toward the periphery of the cells[3]. No eosinophile myelocytes could be found. The curious irregular granules in the

[1] We are inclined to think that the numbers of this variety of leucocyte which are present in normal blood are commonly somewhat underestimated. The reason may possibly lie in the fact that Ehrlich's triacid stain does not bring them out very clearly; Jenner's stain renders them one of the most prominent and unmistakable features in the film. The statement that in health they never exceed 0·5 per cent. is certainly not true.

[2] B. K. Ashford, *loc. cit.* W. L. Blickhalm (*Medical News*, Philadelphia, LXIII. 1893, p. 662) found an excess of eosinophiles and mast-cells (α and γ granulations of Ehrlich), but he does not mention any cell which can be identified as a myelocyte.

[3] Ehrlich and Lazarus (Myers' translation, p. 76) state that, except for tinctorial differences, it is "completely similar" to the neutrophile nucleus. Such a condition is very exceptional. Ehrlich has in another place quoted from Jolly a correct description (*Rapport sur la leucocytose, XIIIᵉ Congrès internat. Méd.*, Paris, 1900, p. 4).

cytoplasm of some of the smaller of the intermediate non-granular cells which stain a metachromatic red with Jenner's stain seemed perhaps rather more numerous in these cases than they do in normal blood.

In spite of the fact that an eosinophilia is occasionally absent, there is no doubt that it may be frequently of service in suggesting the diagnosis of a case of anaemia of doubtful origin. It is a test which can often be easily made when a sample of the faeces cannot be obtained, and the examination of a blood film is a procedure which should always be undertaken in any doubtful case of anaemia. In the present instance it would have cleared up the mystery of " Dolcoath anaemia" years ago. As to its value in diagnosis, where it stands almost by itself, it is not easy to make a definite statement. We have come across four cases (XXII. XXIII. XXX. XLIV.) where the count gave 13·8, 11·2, 22·6, and 13·8 per cent. eosinophiles respectively (for which no other reason could be found) and where very thorough examinations of the faeces failed to reveal the presence of any ankylostoma ova. Two cases gave a history of pit work and illness six years ago which corresponded to those obtained in clear cases of *Ankylostoma* infection at Dolcoath, while the others had been exposed to possible infection, one for several years, the other only recently. It has been shown[1] by the test of autopsies that a few worms may be present in the gut in cases where during life the examinations of the faeces has proved negative. Taking this fact into consideration, there seems to be little doubt that, among a community exposed to infection, a positive blood examination may be regarded as overriding a negative search for ova in the faeces. We have accordingly regarded these four instances as being examples of infection, as well as five other miners (XXVI.—XXIX. and XXXI.) the faeces of whom were not examined, and in whose blood the percentage of eosinophiles varied from 12·6 to 56·2. On the grounds of exposure to infection and an eosinophilia (9·2 to 48·0 per cent.) we also regard the eleven cases given in Section II. of the Appendix as being in all probability infected. Among the pitmen at St Agnes (where the mine is shallow and cool) no history of anything like ankylostomiasis could be obtained except in one case (LXI.): in this instance infection by *Ankylostoma* as a

[1] Leonard Rogers, *Indian Med. Gazette*, xxxv. 1900, p. 129. Bucklers (*Münch. med. Wochenschr.* xli. 1894, p. 21) records an interesting case where no eggs could be found after 227 worms had been expelled with male fern: a further dose however produced 9 more *Ankylostoma*. As P. Sonsino (Davidson's *Hygiene and Diseases of Warm Climates*, 1893, p. 896) points out, eggs can only be found if the intestine contains adult females engaged in oviposition.

cause of the severe anaemia was excluded by the blood-count (3·5 per cent. eosinophiles) and no ova could be found in the faeces. Inspection of one of the shifts as they came up from underground failed to reveal any examples of unusual pallor or of dyspnoea on climbing the ladders, the general healthy appearance of the miners here contrasting strongly with the pallor which is so common at Dolcoath. Blood films were taken from eleven of the least ruddy men who could be found; on examination eight of them showed from 1 to 4·75 per cent. eosins, and two others which were not counted showed no excess of eosins on inspection. The tenth case (LX.) however, who had lately returned from India, gave 23·8 per cent. eosins in the differential count, and a moderate number of *Ankylostoma* eggs were found in his faeces.

It is possible that such cases as XXII. and XLIV. were not infected at the time of examination and that their eosinophilia was a remainder of a previous infection which had died out. We have at present no data by which this question can be settled[1]. The death of a large number of worms is certainly not necessarily followed by an immediate fall in the eosinophiles; indeed in some of our cases the percentage actually rose[2] (see cases I. IV. XI. XL.).

The possibility of the changes which we have described being due in some instances to some other intestinal parasite must of course be considered. The only one of which we obtained evidence was *Trichocephalus*. The characteristic eggs of this worm were found in a good many cases; unfortunately the fact was not noted in all instances but most are recorded in the table in the Appendix. We are not aware that an eosinophilia has at present been established for *Trichocephalus* as it has been for most of the intestinal worms, though a fatal anaemia has been attributed to it.

[1] Ehrlich (*Rapport sur la leucocytose*, p. 11) cites a case which Leichtenstern (*Die Anaemie*, p. 113) has put on record as showing that "after removal of the worm" an eosinophilia (8 per cent.) persists for a long time owing to hypertrophy of the eosinophile part of the bone-marrow. It is however distinctly stated by Leichtenstern that in the following year this same case still harboured a few worms (eosins 8 per cent.), so that there appears to be no ground for assuming either that all the worms were expelled the year before or that a habit of eosinophilia persists after their complete expulsion. In this case, on killing (most of) the worms, the eosins fell at once from 54 to 11 per cent.; from which it might be argued that on killing them all the eosins would have reached a normal figure. The difficulty of being quite sure that any individual is quite free from *Ankylostomata* by any means short of autopsy seems an almost insuperable one. We are inclined to regard the blood-count as the finest test which we at present possess. Thus case IV. is probably still infected, though no ova can be found in the faeces.

[2] The partial digestion and presumable absorption of a number of dead worms might well increase the eosinophiles for a time.

APPENDIX.

TABLE OF CASES.

SECTION I.

	Name and age	History, symptoms, occupation, etc.	Present condition	Ova in faeces	Red cells per cub. mm. in millions	Hb p.c.	Colour-index	Total leucocytes per cub. mm.	Lymphocytes	Intermediate,	Large hyaline	Neutrophile	Eosinophile	Mast-cells
1	J. S., 51	In charge of four shafts (including Engine Shaft) for 18 years. Illness began 3 years ago with general weakness, palpitation and dyspnoea. At home 6 months. On surface 2 years. Some epigastric pain and dyspepsia. Had urticarial bunches often, but not since leaving shaft. Marked general oedema 18 months ago. Has had continual general pruritus for 2 years	Dyspnoea on slight exertion, no oedema, general pruritus	Oct. 31 abundant. *Trichocephalus* present Nov. 14 thymol 264 worms, females 55 p.c. Nov. 15 Nov. 22 ova numerous Dec. 1	3·216	48 / 43 / 43	0·74	8,800	13·4 / 10·8	7·4 / 5·6	6·2 / 3·4	56·2 / 61·0	16·0 / 18·0	0·8 / 1·2
II	J. P., 43	Worked in Engine Shaft. Illness began 4 years ago with palpitation and dyspnoea. At home 5 months, since on surface: has not improved at all. Bowels loose when in shaft, but not since. Had bunches badly, but little pruritus	Dyspnoea on slight exertion, general health bad	abundant Oct. 31. *Trichocephalus* present also worms obtained	2·192	35	0·81	3,800 / Nov. 7	16·6 / 11·8	8·2 / 4·4	5·8 / 2·2	51·0 / 72·0	17·8 / 8·8	0·6 / 0·8
III	M. B., 32 circ.	Machine work. Dyspnoea, palpitation and pruritus 4 years ago. Has worked on surface at stamps for last 4 years; condition has much improved. Bunches preceded illness	Can do work easily	moderate	3·024	38	0·63	6,800	16·2	11·0	10·4	50·0	11·4	1·0
IV	W. F. A., 27	Pitman off Engine Shaft. Ill 4 years ago with dyspnoea, palpitation and cramps in legs. For last 3 years on surface. Had bunches in pit but not since. Has much improved.	A little dyspnoea on exertion, at work regularly	Oct. 31 few. *Trichocephalus* also Nov. 1 thymol 7 worms Nov. 15 no ova Nov. 20 Nov. 25	3·328	37 / 36 / 45	0·56	8,200	16·6 / 21·0	5·2 / 3·0	5·2 / 1·5	53·0 / 39·5	19·4 / 33·5	0·6 / 1·5

		History	Present condition	Worms / date											
V	G. P., 50	In Engine Shaft 21 years. Anaemia and palpitation in 1897. "Pimples all over" 4—6 weeks before palpitations. Has improved greatly	Well except for slight dyspnoea, works underground	moderate. *Trichocephalus* present	3·968	56	0·71	7,500	16·6	11·8	7·8	47·0	16·8	0
VI	B. R., 20	Eastern Shaft. 3 years ago went down Engine Shaft and immediately had rash on back, shortly afterwards began to be pale; has not improved since. Palpitations, little dyspnoea, intermittent diarrhoea	Too ill to work but can get about, has general pruritus	abundant	3·376	54	0·79	13,100	6·6	13·4	15·6	43·0	20·6	0·8
VII	W. P., 28	Machine man. 2 years ago pale and short of breath. At home 4 months, never ill till in Engine Shaft. Bunches appeared before anaemia	Slight dyspnoea but feels quite well, pale	moderate	4·424	74	0·83	10,750	26·0	6·2	7·0	54·4	6·0	0·4
VIII	J. P., 34	Has never had any symptoms at all. Machine man, has worked all over the mine exactly as Case VII.	Has exceptionally healthy appearance	few	4·712	94	1·00	12,700	31·8	9·6	5·0	36·0	17·2	0·4
IX	A. T., 36	Pale and palpitations in 1896, ill till 1901. Has gone underground about 2 days a week throughout, and (except when too ill) up to the present time. Botches several times	No symptoms now present	very few	4·936	102	1·04	10,000	31·4	4·0	3·6	55·2	5·6	0·2
X	N. S., 17	12 months underground at Tincroft, at Dolcoath 12 months. 7 months ago was in Engine Shaft for 4 months. Ill for last 2 months with dyspnoea, some epigastric pain, botches	Dyspnoea on exertion, very pale, cannot work	moderate	3·432	40	0·58	Nov. 15 24,400; Nov. 19 31,400	10·0	7·6	4·2	48·4	29·0	0·8
XI	R. C., 25	Came from E. Pool to Dolcoath for 4 days 18 months ago (not to Engine Shaft); since at E. Pool. Ill 12 months with pallor and dyspnoea; at home 7 months, and confined to bed 14 days. No skin affections	In bed, very weak and intensely pale, no oedema	Nov. 16	1·533	17	0·56	12,960	14·2	2·4	3·6	62·8	15·8	1·2
			Ova abundant, thymol Nov. 21, 28, and Dec. 19, many worms; ova still numerous on Dec. 10	Dec. 10	2·192	25	0·57	12,200	7·4	2·6	3·2	74·4	11·0	0·4 neutrophile myelocytes 1 p.c.
			Able to go for a walk on Dec. 16 *Trichocephalus* ova also present	Dec. 18 / Dec. 20		36		7,690 / 7,850	18·4 / 11·0	1·8	5·2 / 5·2	58·0 / 67·0	17·0 / 13·6	1·4 / 1·4 neutrophile myelocytes 0·2 p.c.

Section I. (continued).

Name and age	History, symptoms, occupation, etc.	Present condition	Ova in faeces	Red cells per cub. mm. in millions	Hb p.c.	Colour-index	Total leucocytes per cub. mm.	Lymphocytes	Intermediate	Large hyaline	Neutrophile	Eosinophile	Mast-cells
XII	J. C., 17	Between New East and Valley 3 months, previous in Harriet for 18 months, pale and short of breath 6 months	At work underground, does not look very pale	moderate Nov. 20, Nov. 21, Nov. 24	3·192	50	0·78	44,000	6·3, 8·9, 9·0	0·5, 3·6, 3·1	0·7, 0·5, 0·5	19·5, 20·7, 20·7	72·7, 65·8, 66·2	0·3, 0·5, 0·5
XIII	T. T., 32	4 years in Engine Shaft. Has suffered from bunches ever since he came to mine (has furuncles now on both ankles and left elbow). No dyspnoea or palpitation	Looks pale but feels quite well	moderate	3·768	47	0·63	16,200	16·8	3·6	3·0	61·6	14·6	0·4
XIV	J. C., 22	Works at E. Pool. Has been to Dolcoath with machines several times, last 8 months ago. No botches. No dyspnoea or palpitation	General health not very good	very few. *Trichocephalus* present	3·330	92	1·38	13,500	20·6	3·4	1·6	45·8	27·6	1·0
XV	J. M., 25	Engine Shaft 8 years. A little dyspnoea. Not pale. Botches frequent	At work, slight dyspnoea	moderate	4·072	58	0·71	13,500	9·2	8·0	8·0	52·0	22·0	0·8
XVI	R. S., 21	Worked in S. Lode 4 years ago. Became pale and short of breath and gave up mining. Has been in butcher's trade since. Much better but not quite well. Never in Engine Shaft. No botches	Working, does not look anaemic	very few. *Trichocephalus* ova also found	4·488	99	1·11	6,200	36·6	6·6	3·2	40·2	12·0	1·4
XVII	J. R., 27	In Engine Shaft till 2½ years ago, since in Eastern. Pale, etc. 3 years ago; in hospital for a month	At work underground	moderate	4·128	74	0·90	12,400	27·6	18·0	6·6 (300)	43·6	3·7	0·3
XVIII	J. M., 18	Began in Engine Shaft 2 years ago. Ill for 9 months on and off work for 2 months with palpitation and dyspnoea. Bunches preceded paleness. Almost constant diarrhoea	Cannot work	moderate	3·352	36	0·53	10,700	17·5	6·5	2·8	42·5	30·1	0
XIX	J. R., 25	Engine Shaft 12 months, previously all over mine. Ill 12 months with dyspepsia. No dyspnoea. Not paler. Bunches occasionally	At work, does not look anaemic	few	3·456	70	1·01	9,250	25·0	6·4	4·4	53·0	10·4	0·8

		History	Condition											Worms
XX	R. W., 29	Engine Shaft 2 years. Ill 18 months with epigastric pain and vomiting. Has had botches	A good deal of dyspnoea on exertion	5·384	62	0·58	9,000	37·8	7·4	2·8	38·6	12·2	1·2	few
XXI	F. N., 36	At Dolcoath 3 years, previously at Tincroft and abroad. Has been off work 5 months with general weakness. Sometimes short of breath. Very few botches	Cannot work, possibly has tubercular peritonitis	5·280	70	0·66	8,650	19·5	15·8	6·8	37·3	20·6	0·16	moderate
XXII	J. W., 49	Pale and dyspnoeic 6 years ago. Has not been underground for 6 years, but has worked as a fitter. Does not handle anything from mine	Pale, general health bad	3·272	89	1·37	7,600	31·0	3·8 (6·00)	2·8	48·2	13·8	0·4	none found on 2 separate occasions
XXIII	A. R., 22	Carpenter. Has worked in upper part of Eastern Shaft for last 9 months only. A little paler lately. No dyspnoea. Marked constipation with colicky attacks for last 3 months	Looks very well	4·848	88	0·91	8,100	33·0	4·2	3·8	47·6	11·2	0·2	none found
XXIV	S. J., 24	Underground, not in Engine Shaft. A little palpitation, no dyspnoea. Has become a little paler. Has had bunches (urticarial) several times, first attack 9 months ago	Feels quite well	3·144	46	0·74	12,300	10·6	9·0	5·4	42·8	32·0	0·2	fairly numerous
XXV	W. J. S., 29	In Engine Shaft 7 years. Pallor and dyspnoea for 5 years. A good deal of epigastric pain. Had no bunches till 3 or 4 years ago	At work, a little dyspnoeic, very pale	3·216	46	0·72	12,000	13·2	4·8	4·6	57·6	19·0	0·8	moderate : very 4 worms+
XXVI	J. M., 22	New East underground. No dyspnoea. A little paler lately. Small bunches occasionally	Feels quite well	4·080	80	0·98	11,100	20·2	4·2	6·8	55·6	12·6	0·6	
XXVII	M. T., 50	At Dolcoath 2 years in Engine Shaft. No dyspnoea or palpitations. Diarrhoea for last 5 weeks	Looks very pale but feels fairly well	3·296	44	0·67	11,600	19·0	4·2	3·8	55·6	16·6	0·8	
XXVIII	J. S., 24	Pitman. A little dyspnoea and palpitation, and rather paler. No botches	Looks very well	5·112	100	0·98	6,900	23·8	4·4	3·8	47·0	20·4	0·6	
XXIX	E. J. T., 18	Underground 12 months stoping. For last 6 months has had epigastric pains and is a little paler and slightly dyspnoeic. Botches sometimes	At work, looks quite well	4·208	98	1·16	20,600	21·0	4·2	1·2	16·6	56·2	0·8	

SECTION I. (continued).

Name and age	History, symptoms, occupation, etc.	Present condition	Ova in faeces	Red cells per cub. mm. in millions	Hb p.c.	Colour-index	Total leucocytes per cub. mm.	Lymphocytes	Intermediate	Large hyaline	Neutrophile	Eosinophile	Mast-cells
XXX J. C., 22	Engine Shaft 5 months. Stoping previously in New East for 8 years. A little dyspnoeic for 3 years. Not paler. Bunches sometimes	At work, does not look anaemic	no ova found	4·176	82	1·00	13,600	14·8	7·0	3·6	51·8	22·6	0·2
XXXI P. C., 29	Underground in Engine Shaft till dyspnoea and palpitations made him come on surface 4 years ago. Constant diarrhoea for about 5 years (no melaena)	At work		4·706	66	0·70	10,500	14·2	5·5	4·0	60·25	15·25	0·75
XXXII S. C., 23	Stoper in Eastern Shaft. Never worked in New Sump. Weak and sick for last few weeks. Abdominal discomfort. No dyspnoea. Poor appetite. Has had bunches all over	At work	moderately abundant	5·350	80	0·75	56,000	5·4		2·8	25·6	66·2	0
XXXIII J. H. S., 18	Underground at Dolcoath 4 years. Pallor and dyspnoea on exertion 2 years ago, now better. Considerable epigastric pain. Had bunches when working in Engine Shaft		moderate	2·900	38	0·65	6,700	27·0	15·0	7·0	36·5	14·0	0·5
XXXIV J. M., 34	Machine man 6 years. Pale and dyspnoeic for last 12 months		moderate		88		7,200	12·8	11·2	4·0	52·0	20·0	0
XXXV C. M., 36	Engine Shaft. Pale and dyspnoeic in 1897. At home 12 months, on surface since. Botches began just before palpitation. Intermittent diarrhoea	Can work, is in-definitely ill	very few		70			15·6	6·4	3·4	47·4	26·4	0·9
XXXVI T. S., 52	Underground 30 years. Pale and a little short of breath 6 years ago, is now better. Not in Engine Shaft	Can work without difficulty	moderate		58			21·6	3·0	1·4	69·8	3·6	0·6
XXXVII W. J.	Underground superintendent. Constantly in Engine Shaft. Became very pale 2 years ago but had no palpitation or dyspnoea	Appears to be in perfect health and feels very well	moderate		104			41·2	4·6	2·4	34·2	17·0	0·6

XXXVIII	J. W., 50	Underground. Pale and short of breath 2 years ago. Has kept the house since March 1901, 6 miles from Dolcoath. Had many (furuncular) bunches when in mine	Has fibrosis of left lung, short of breath on exertion, often has attacks of urticaria	moderate: 111 worms, females 75 p.c.	34	15·6	3·0	1·4	55·6	24·4	0
XXXIX	T. W., 19	Underground New East. Paler and a little dyspnoeic for 18 months. Botches frequently, but not lately	Works underground without difficulty, looks very pale	many	36	19·0	5·2	1·6	54·0	20·2	0
XL	F. C., 22	Underground Engine Shaft 3 years. A good deal of dyspnoea. Not paler. Botches sometimes	Does not look anaemic	rather abundant Dec. 12 thymol Dec. 19	52 / 62	14·2 / 15·6	5·2 / 4·0	1·8 / 5·2	69·0 / 58·0	9·4 / 16·4	0·4 / 0·8
XLI	T. U., 49	Underground machine man. Came to Dolcoath 6 years ago, and soon after had shortness of breath and became pale. Had bad attacks of haematemesis 6 years and 9 months ago	Pale, a little dyspnoeic on exertion	moderate	50	15·6	6·4	3·0	58·4	15·6	1·0
XLII	J. W., 47	Underground in Harriet 10 years. Pale and short of breath 2 years ago, better now. Bunches frequently	Works easily, but general health indifferent	moderate: a few worms	61	12·8	7·6	3·8	64·8	10·2	0·8
XLIII	A. B., 21	Harriet 6 months, previously in Engine Shaft. No dyspnoea or palpitation. Is rather paler than formerly. Has furuncles on knee now	At work, no symptoms of anaemia	many	66	13·6	10·2	5·8	62·4	7·0	1·0
XLIV	C. T., 37	Underground in South Lode. Very pale in 1894. At home 2½ years and on surface since. No history of "botches." Frequent diarrhoea	Does not look anaemic. Epileptic	none found		27·0	9·0	1·8	48·0	13·8	0·4
XLV	W. C., 48	At Dolcoath 24 years, except 1894–1900 in India. Some dyspnoea on exertion for last 5 months	Has early fibroid changes in lungs	few	100	8·0	7·0	4·75	67·25	11·5	1·5
XLVI	R. B., 44	30 years underground at Dolcoath. Three attacks of pleurisy recently	Pale, at work underground	moderate		20·0	5·5	5·5	61·0	13·0	0·5

Section II.

Containing eleven cases of underground workers at Dolcoath whose faeces were not examined but whose occupation raises a strong presumption that they are infected though they shew little or no symptoms. The blood examinations are incomplete but the differential count greatly strengthens this presumption.

	Name and age	History, occupation, etc.	Present condition	Hb p.c.	Lymphocytes	Intermediate	Large hyaline	Neutrophile	Eosinophile	Mast cells
XLVII	A. G., 19	Underground at Dolcoath. Considerable epigastric pain. Palpitation on exertion. Not paler : bunches occasionally	Works easily underground	80	15·8	6·8	3·6	50·0	23·0	0·8
XLVIII	J. C., 22	Engine Shaft 2 years. Palpitation 5 months. Not paler. Epigastric pain. Botches sometimes	,,	92	19·0	5·4	2·6	56·2	15·2	1·6
XLIX	R. H., 36	21 years underground. Engine Shaft 6 months. No symptoms. A few botches	,,	99	19·3	8·3	3·7 (3·00)	50·0	17·3	1·3
L	A. M., 22	Underground 7 years. At Dolcoath in Engine Shaft 7 weeks. No symptoms	,,	98	21·75	7·25	3·75 (4·00)	54·5	12·5	0·25
LI	H. R., 35	Underground in Engine Shaft 2 years. No symptoms	,,	86	15·6	6·8	0·8	44·8	31·0	1·0
LII	R. E. C., 22	Underground in Engine Shaft 18 months. A little dyspnoea. Botches sometimes	,,	71	12·4	7·0	4·4	28·0	48·0	0·2
LIII	C. T., 52	Underground for many years. Has been to India, etc. Has never had any anaemic symptoms. Has always been very subject to attacks of urticarial botches at Dolcoath	,,	90	16·0	13·75	8·25 (4·00)	43·25 (4·00)	18·0	0·75
LIV	E. L., 29	Machine man. Has miner's phthisis	,,	59	6·0 (combined)		5·8	73·4	11·4	0·8
LV	H. V. T., circ. 25	Surveyor underground at Dolcoath 5 years. No history of anaemic symptoms	Feels quite well	85	16·0 (combined)		5·5	59·0	19·5	0·5
LVI	S. H., 21	Works in Engine Shaft. Bunches and paler lately	Works easily underground		19·5 (combined)		5·0	56·0	17·0	2·5
LVII	J. R., 59	Underground at Dolcoath till palpitations and dyspnoea made him leave 3 years ago. Improved and worked at South Condurrow till recently	Shoemaking now		16·6 (combined)		5·6	67·8	9·2	0·8

SECTION III.

Surface workers at Dolcoath in whom there is no suspicion of infection.

	Name and age	History, occupation, etc.	Present condition	Hb p.c.	Lymphocytes	Intermediate	Large hyaline	Neutrophile	Eosinophile	Mast cells
LVIII	R. P., 54	Carpenter. Does not handle anything from underground and has not been down mine for 30 years. Always been very well	In excellent health	105	33·6	3·6	3·4	54·4	4·8	0·2
LIX	R. G., 60	Underground till 9 years ago. No history of any symptoms. Works at tin-dressing	Enlarged prostate and cystitis		7·0	4·4	5·4	81·6	1·2	0·4

SECTION IV.

Underground workers at West Kitty Mine, St Agnes (see p. 103).

	Name and age	History, occupation, etc.	Present condition	Hb p.c.	Lymphocytes	Intermediate	Large hyaline	Neutrophile	Eosinophile	Mast cells
LX	J. J.	No history or symptoms of anaemia. Has lately returned from 4 years in the Mysore Gold Mines. In faeces a moderate number of Ankylostoma and abundant Trichocephalus ova found			14·6	6·0	3·6	50·8	23·8	1·2
LXI	G. C., 58	Very weak and anaemic 8 months, in bed 4 months. No fever or local symptoms. Possibly malignant. No ova found in faeces. Blood does not shew any blood disease		35	8·0	4·5	2·0	81·5	3·5	0·5
LXII to LXIX	G. R.	Eight pitmen. No history or symptoms of anaemia in any case. All in good health. Differential counts of 200—300 only			39	8·5	3·5	46	1	2
	J. C.				26	4·3	3·3	65·3	1	0
	W. F. P.				18	11·5	5·5	61	3·5	0·5
	W. W.				15	8·5	6	69·5	1	0
	W. M.				38·7	12·7	3·3	42·3	3	0
	W. R.				18·0	16·5	7·5	56	2	0
	J. H.				31·5	5	2	56·5	4	1
	W. T.				27	4·75	2·75	60·0	4·75	0·75

(2 normoblasts in counting 100)

WEST.

EAST.

SECTION OF DOLCOATH MAIN LODE.

Fig. 1 (see p. 97).

Scale 128 Fathoms to an inch

Copper workings

Copper and Tin mixed

Tin workings

West Dolcoath is partially
worked to a depth of
280 fms under adit
and is drained by
Dolcoath to the 140 fms.
level

Harriett downcast to 160, the lower
workings being upcast
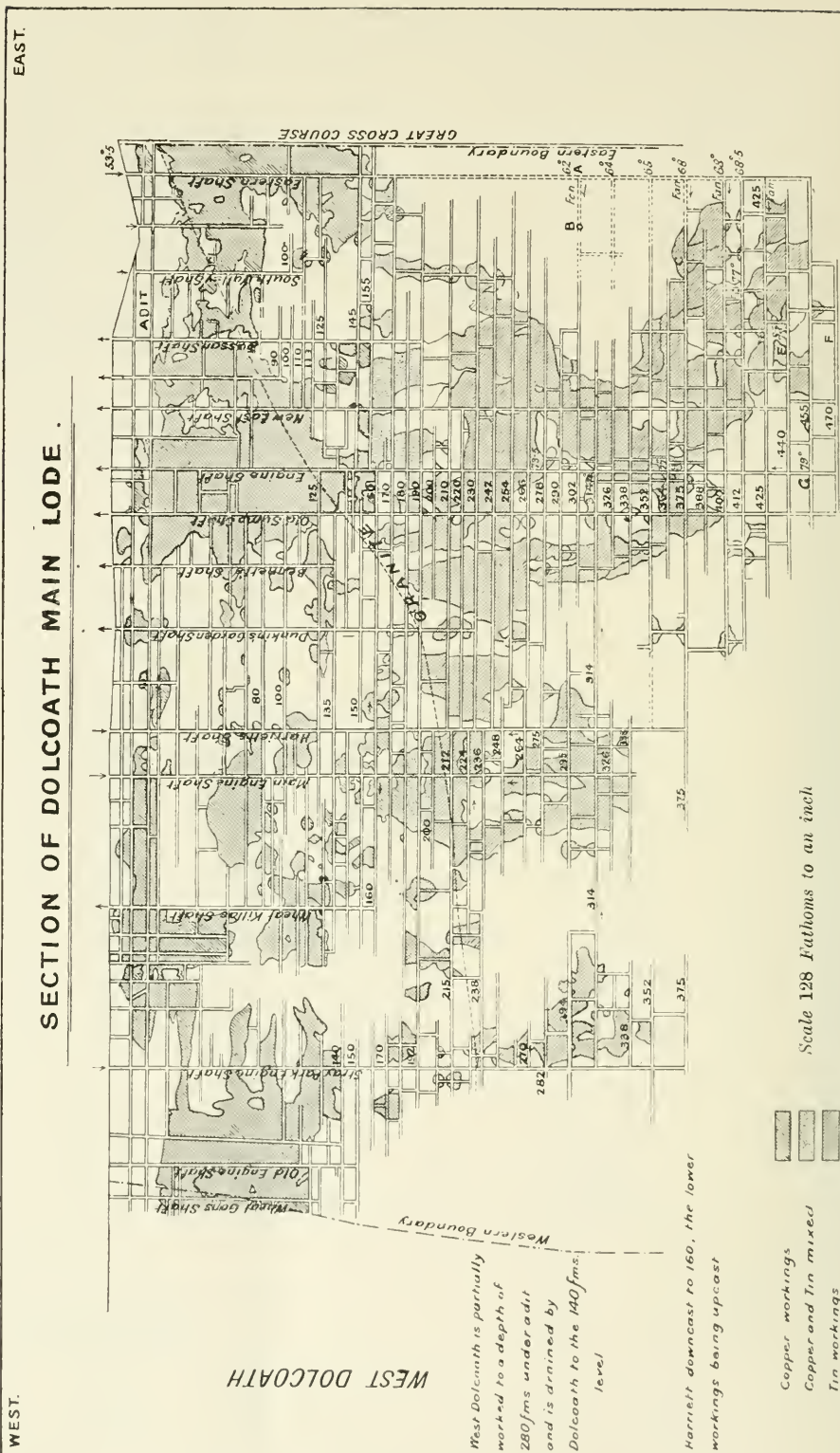
NOTE ON THE CO-RELATION OF SEVERAL DISEASES OCCURRING AMONG ANIMALS IN SOUTH AFRICA.

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In South Africa one finds quite a number of ailments occurring in the domesticated animals, and while the onset, symptoms, and morbid anatomy of those affected show the ailments to be peculiar to Africa, the names given to the maladies are unknown in any other country.

Horses and mules are liable to the enormously fatal malady known as Horse-sickness. The mode of onset, symptoms, and morbid anatomy of this disease have been already fully described[1].

High-bred goats and sheep are exceedingly liable in certain areas of Cape Colony to a disease known as Heart-water. The areas within which this disease exists begin at the coast in the Eastern Province and extend inland irregularly for some distance.

In the animals which die of this disease it is common to find a considerable quantity of pale yellow serous effusion in the pericardium. The epi, myo and endocardium commonly show little or no departure from the normal. The pleural cavity may contain some yellow serous fluid or may be empty. The lungs may be almost normal or there may be some exudation found in the interlobular tissue. While the lungs as a whole may be quite pale in colour there may be found irregular, sharply defined, chocolate-coloured patches of congestion. There may also be found more or less of gastro-enteritis, and in cases running to the full term the gall-bladder may be distended with more or less inspissated bile, which, while usually of a deep bottle-green, may be sometimes brown in colour.

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[1] *Proc. Royal Society*, Vol. 67. *Reports of the Director of the Col. Bact. Inst.*, 1894 and 1899.

The incubation period after the intravenous inoculation of a clean goat with 5 c.c. to 30 c.c. of the blood of an animal dying of the malady is as a rule about ten days. This period, however, may be greatly diminished or extended; from a few days up to nearly three weeks in some cases.

From the point of view of experimental infection, the results which I have obtained from experiments conducted on nearly five hundred goats are paradoxical in the extreme. When I first began my investigations I used goats which were born and reared on Mr Palmer's farm, near Grahamstown, which was believed to be outside of the area infested with the disease. On infested farms the mortality during the summer season is very high, but no unnatural mortality had occurred on this farm during the past ten years at least.

The material used for purposes of infection was the blood taken from animals dying of the disease at Koonap and at Somerset East. The blood was either simply defibrinated or mixed with a small quantity of a solution of neutral citrate of potash. I was unable to find that either had any advantage over the other as an infecting agent. Subcutaneous injection of doses as large as 40 c.c. almost always failed to produce death, although some oscillation of the temperature of the inoculated animals was observed. Intravenous inoculations of doses up to 30 c.c. were uncertain. Where the animals inoculated in this way developed the disease and died there was no certainty that their blood would produce the virulent disease in others. Failures have occurred even with the injection of 100 c.c. into the jugular vein. In some cases blood which was drawn from inoculated animals, which did not themselves die, proved capable of setting up the virulent disease in others.

As further indicating the paradoxical nature of the malady I may add that in by far the greater number of these goats which had resisted inoculation it was proved that an inoculation of even a much smaller dose of blood at a later date, or exposure of the animals in an infested veld, was attended with the production of the malady, followed by death.

I felt, therefore, that these goats, which largely resisted infection although being not immune, had acquired what one might term a modified resistance or acclimatization.

This is the more probable from the following circumstance. Mr Thomas Hoole, a well-known breeder at Highlands, made a purchase of a considerable number of goats from a district of Somerset where

the disease is known to be absent. After purchasing he sold part to Mr L. White, whose farm lies many miles distant. After these goats had been placed on the respective farms they began to die of heart-water, while contrarily the animals belonging to the place did not die. On both farms heart-water occurs.

I may further add that the ordinary Boer goat is practically insusceptible to the malady, and that the pure bred Persian goats possess a high degree of insusceptibility to natural infection.

In some parts where the disease only occurs to a slight extent I have had it reported to me by the farmer as being gall-sickness; thus called as the gall-bladder is often very much distended with bile.

Since that time I have imported all goats by train from a clean area in Somerset, and in these animals I have found it much easier to keep up a strain of infection from animal to animal. Still, however, inoculation frequently fails, and I felt constrained to report to my Government that goats evidently were not the proper animal host for the contagium of this malady. In sheep the conditions are practically the same.

In cattle a number of names are applied to diseases by the farmers, which have given me immense trouble in the attempt to identify. The names with which I shall now deal are:

1. Imapunga (Kafir: " Lung ").
2. Boschziekte (Bush-sickness).
3. Gall-sickness.
4. Veld-sickness.
5. Black Lung-sickness.
6. Rivierziekte (River sickness).

There is no official work which enables anyone to identify the maladies above named, but since my own work has been completed I have obtained, a few weeks ago, a copy of the "Report of the Commission appointed to inquire into Disease in Cattle in the Colony," dated 1877. Among the numerous minutes of evidence I desire in particular to refer to the very valuable and strikingly accurate observations of the late Mr J. Webb, who owned then a farm near Grahamstown, consisting of sour veld as contrasted with the sweet veld of the Karoo.

" Question 2705. Have you noticed any change in the veld during the last few years?

" Yes, stock of all kinds have been doing badly, and sheep and goats it is now impossible to keep on farms which at one time were considered to be the best grazing farms in this neighbourhood.

"2706.   What do you think is the cause of it?

"My opinion is we have a tick which made its appearance in the last eight or nine years, I suffered from them then, a bonte-tick, small, like a ladybird.   I was farming on a farm without ticks; directly this tick appeared all my stock did badly, calves died of gall-sickness, boschsickness, one man lost 2000 or 3000 sheep and goats, I believe the tick caused it.   I have also shot bush-bucks suffering from the same causes, this was at Southey's Poort, Fish River.   As this tick increases so diseases increase, for wherever the tick is found there are the same diseases; the tick has now travelled over 60 miles.

"2710.   Did you open and examine them?   A few sheep, not often.

"2711.   What did you notice?   The heart-bag and chest full of water.

"2716.   You have had large experience in cattle?   Yes.

"2717.   What do they die of?   Below Grahamstown of gall-sickness. North very few die compared to the south.   We have three sicknesses here, called by the farmers: gall-sickness, bosch-sickness, and sweet veld-sickness, I believe they are all the same."

It is generally known to farmers that if Karoo cattle are brought down to the coast areas of the Eastern Province the greater number will die.   For restocking the northern territories large numbers of cattle have been bought, of which great part are Karoo animals.   Of these many have been grazing on the same farm which Mr Webb spoke of, and of the Karoo cattle a very large number are already dead.

Horse-sickness co-related to Veld-sickness.

During the earlier part of my work in this colony I endeavoured without success to transfer the disease known as horse-sickness from the horse to cattle.   The cattle used in these experiments were of the class known as Zuurveld (sour veld) cattle.

It has been known during several generations of farmers that if cattle living in sweet veld areas are brought to Zuurveld areas they are exceedingly liable to die very soon after their arrival.   Owing to this the Zuurveld cattle, sold on the Grahamstown market, fetch higher prices than those from sweet veld, and, indeed, most farmers in this area refuse to purchase sweet veld cattle at any price owing to the area being a Zuurveld one.   If then sweet veld cattle die when transferred to sour veld, what is the nature of the disease produced in them?

After inoculation for Rinderpest had been well advanced in the

Eastern Province it was found necessary to be exceedingly careful of the kind of animals used to produce virulent blood, and a large number of animals were conveyed from sweet veld areas to a camp at Waai Nek, about two miles from this Institute. Of these considerable numbers died, but the cause of their death was not understood, and the enormous pressure of work connected with Rinderpest prevented definite investigations being taken up for this purpose. We had to content ourselves with attempting, by treatment, to save as many as possible.

Most of the deaths were reported to me by Mr Robertson as belonging to Steynsburg cattle and he emphasized the fact of their being sweet veld cattle while our veld was Zuurveld.

While this condition of things was in progress, a Bechuana boy (a herd brought from Taungs who had worked with me there) living at the camp, came in and reported to my veterinary assistant, Mr William Robertson, M.R.C.V.S. (now assistant to the Colonial Veterinary Surgeon), that one of the cows had died of " Paardeziekte." As a result of this report Mr Robertson rode to the camp and returning almost immediately stated to me that an animal had just died and that it had a cloud of white foam lying around the nostrils and mouth. I immediately proceeded with him to the camp and saw the animal lying dead. It had a large quantity of white foam lying around the nose and mouth, exactly as one sees so frequently in the cases of horses which have died of horse-sickness.

On making a post-mortem examination the similarity to horse-sickness was extended, since we found the following conditions:

The lungs showed an exceedingly well-marked interlobular yellow serous exudation. This was so characteristic, that, had the lungs only been shown to me, I should have believed they had been taken from a horse that had died from horse-sickness. In another case of the same sort which had been dead some hours before the post-mortem examination was made, there was in addition some emphysema of the apices and free edges of the lungs. The pericardium contained an excessive amount of yellow serous fluid. No abnormal condition was seen in the abdominal cavity except the spleens, which were slightly enlarged, the liver, which was in both cases congested and friable, and some exudation of serous material into the omentum and mesentery. No micro-organisms of any kind were found in the blood.

This occurrence was somewhat surprising to us both and I thereupon determined upon attempting once more the infection of clean cattle

with horse-sickness from the horse. Accordingly I took several animals of a new consignment to the Institute and then under careful conditions carried out the experiments.

On the 4th February, 1898, Mr Robertson and I inoculated a clean young ox with 30 c.c. of fresh horse-sickness blood which we injected into the jugular vein. Some reaction occurred during the first few days after which the temperature fell, but on the 16th day it rose. The temperature maxima were as follows till the moment of death.

16th day	...	106·4° Fahr.	19th day	...	107·2° Fahr.
17th „	...	106·6° „	20th „	...	106·2° „
18th „	...	107·0° „	21st „	...	died.

"The post-mortem was of interest inasmuch as nearly every symptom of horse-sickness was reproduced, the interlobular pulmonary effusion, the pleural and pericardial effusions[1]."

Ten c.c. of the blood of this ox was used to inoculate by subcutaneous injection horse No. 122. After an incubation period of eight days the temperature rose, and the animal died, on the 13th day, of typical horse-sickness.

Ten c.c. of the blood of this ox was also used, by intravenous injection, to inoculate a second ox, in which the temperature rose to 106·4 on the 11th day, to 107·4 on the 12th, and which we killed when dying of the disease on the 16th day.

At that time, having succeeded so completely in transferring the disease to cattle, I tried also to infect goats. The goats, however, were born in this area and are more insusceptible than goats taken from other areas. Of the several goats inoculated none died, but most had severe reactions. One of these goats, which had been inoculated with 10 c.c. subcutaneously of preserved horse-sickness blood, and developed a high temperature as a result, was bled on the 10th day after inoculation.

A young ox was inoculated with 30 c.c. of this blood by intravenous inoculation on the 18th February, 1898. During the first few days the temperature was irregular and then took a normal course, but thereafter the following temperatures were recorded.

10th day	...	104·6° Fahr.	14th day	...	107·4° Fahr.
11th „	...	106·6° „	15th „	...	107·4° „
12th „	...	106·6° „	16th „	...	105·4° „
13th „	...	107·0° „	17th „	...	96·8° death.

[1] *Vide Report of the Director of the Col. Bact. Inst.* 1898.

In this case the post-mortem examination showed very well-marked symptoms of heart-water.

These experiments therefore showed :

(1) In a most definite fashion, that cattle, from sweet veld areas are more or less susceptible to horse-sickness.

(2) That the disease so produced was indistinguishable from that which had occurred spontaneously in our camp.

Still, however, I was unable to identify the disease, although I learned that it was well-known to the Kafirs under the name of Imapunga.

Shortly after this a number of deaths were occurring among young calves on the farm of Mr Hyde, and Mr Robertson and I who proceeded there obtained a post-mortem examination which enabled us to determine that it was the same disease which we had already seen in our camp·

During the past two years a very large mortality has occurred among young calves from this disease, but it is to be remarked that the old animals are either insusceptible or well-protected, since very few of the old animals, which have been accustomed to the veld, die of it.

In the case of animals, however, which are brought from sweet veld areas it is the rule rather than the exception for them all to die. I have had to import a considerable number of calves from other areas for use in the Institute, and among these a fairly heavy mortality has occurred from this cause when they have been allowed to run in the veld.

During the war a large number of fine trek oxen were sent to Grahamstown by the military authorities, and to the best of my knowledge almost all of these coming from De Aar, Naauwpoort, and Cradock eventually died. The following will show the results in two lots, coming respectively from Naauwpoort and Cradock.

(1) Oxen from Naauwpoort (16) which arrived at Grahamstown on the 23rd August, 1901.

1 died on Sept. 25th			1 died on Oct.	8th		
1	„	„ 27th	1	„	Nov.	15th
1	„	Oct. 3rd	1	„	„	24th

(2) Oxen from Cradock (14) arrived at Grahamstown on 2nd December, 1901.

1 died on Dec. 24th			1 died on Jan.	7th, 1902		
1	„	„ 25th	1	„	„	11th, „
1	„	„ 30th	6	„	„	12th, „
1	„	Jan. 2nd, 1902	1	„	„	13th, „
1	„	„ 3rd, „				

I had an opportunity of examining some of these animals and was able to determine the identity of this disease with that which I had formerly seen occurring spontaneously and with that which I had produced by the inoculation of clean animals with horse-sickness blood.

While the Kafirs call this by the term Imapunga, I have found, by consulting transport riders whose experience extended over many years, that this disease is known to them under the name of Veld-sickness or Veldziekte.

The principal lesion is an exudation of a yellow serous fluid into the following structures :

(1) Subcutaneous : in and along the lines of the intermuscular fasciae.

(2) Sometimes but not always in the pleural cavity.

(3) Commonly into the interlobular tissue of the lungs. Sometimes it is present to an exceedingly slight degree here, and it is necessary to examine carefully to determine where the normal becomes abnormal since the interlobular tissue in ruminants is more lax than in the equids. In very many cases, however, one finds the interlobular infiltration forming bands from one-eighth to a quarter of an inch in thickness.

(4) Into the pericardium. The amount found in this situation varies within wide limits : in some cases it is but little in excess while in others the pericardial sac is filled. A variation is to be found also in horse-sickness : I have found in some horses only a few ounces of fluid while in others more than half a gallon was found in the sac.

(5) Around the base of the heart.

(6) In the anterior mediastinum.

(7) Between the lower border of the pleura and the diaphragm. I have several times found the exudation to form here a solidified layer nearly half an inch in thickness.

(8) Into the tissue of the omentum and mesentery.

(9) Into the submucosa of the intestines.

Secondary lesions :—(1) Collapse of lobules of the lung with a corresponding traumatic emphysema of the adjoining lobules.

(2) In cases which live for a day or two longer than the more highly susceptible animals it is common to find congested lobules of a dark, almost black colour. These lobules are sharply defined from those immediately adjoining, and from their somewhat superficial resemblance to the appearance seen in pleuro-pneumonia such cases are called by the farmers black lung-sickness.

(3)   In some cases one finds extravasations of blood below the endocardium of the left ventricle, especially in relation to the attachment of the chordae tendineae.

(4)   The liver is commonly congested and enlarged, and in the last stages the gall-bladder is distended.   The bile is of a deep green colour as a rule, but in some cases is brown.   When the quantity of bile is very small it may be of a somewhat syrupy consistence, but never shows the peculiar tenacious mucous character so well known in Texas fever.

(5)   The small stomach is frequently the seat of patches of congestion, more or less of a red colour, which may even have gone on to active inflammation.

(6)   The conditions seen in the small stomach may be found frequently in the intestines, and a general gastro-enteritis may even be set up.

(7)   The spleen may be slightly enlarged but is firm in consistence. The malpighian bodies are more prominent than in the normal condition.

(8)   A slight amount of yellow serous exudation may be found sometimes in the pelvis of the kidney : otherwise the organ is normal.

(9)   In even the best marked cases the urine and the bladder are commonly absolutely normal.

(10)   In cases which have been dead for some time and exposed to a hot sun there may be some patches of emphysema in the lungs.

On examination of the blood and of smears from the kidneys and liver no micro-organisms are to be found except in animals which have been dead for some hours, when a large putrefactive bacillus is frequently to be found.   The blood is always of a good colour and the rapidity of coagulation is always increased.

The fever in these cases is commonly very high.   A remarkable feature in the malady is the fact that animals may seem in perfect health, yet when the temperature is taken it may be found to be over 106° F.   It is common to find animals showing symptoms of illness only a few hours before death.

As this disease is well defined in cattle and runs on parallel lines with horse-sickness in horses, I suggest that it should be denominated "South African cattle-sickness."

While the blood of the first ox proved virulent to an ox I found after three transferences through oxen that it becomes relatively virulent to the ox but may fail to produce virulent disease in the horse even when used in the fresh state.

Heart-water.

In my Annual Report for 1896 observations were made which at a later date were communicated to the Royal Society (Vol. 65) showing that the germs of Red-water or Texas fever may remain latent in the blood of cattle for long periods of time after their recovery from an attack of the malady, and that cattle born on red-water veld although they may not have been affected by this malady yet can and do carry infection, in a latent form, in their blood.

During my investigations into heart-water I began to have suspicions that something of the same nature was concerned in regard to the latter malady.

The following experiments show in how far these suspicions were verified.

Experiment 1 : To prove that the contagium of heart-water may be communicated to a susceptible animal in a non-virulent form and passed in succession through several others, eventually being raised to full virulence in the passage : I obtained a number of clean goats by train from a clean area and enclosed them in a court-yard which, in turn, was bounded by stone walls. Along one side of this yard galvanized iron enclosures were erected into which the animals were placed while under experiment. The most rigorous care was exercised in regard to cleanliness of the place, each shed being at frequent intervals thoroughly cleaned and disinfected.

While I have found that horse-sickness blood can be preserved, by the addition of an equal volume of glycerine and water containing 1 per 1000 of phenol, so that it retains its virulence for at least three years, the blood of goats dying of heart-water when so treated loses its virulence in a few days' time. Such preserved blood does, however, almost always set up a slight oscillation of the temperature in animals inoculated with it.

Goat No. 252 was inoculated with 10 c.c. of glycerinated blood taken from an animal which had died of heart-water. The injection of this material was made subcutaneously on September 5, 1901. No febrile change was observed until September 21st. On this day the temperature rose to 107° F. in the middle of the day but regained the normal on the following day.

Goat No. 258 was now inoculated with 100 c.c. of the blood of No. 252. Some irregularity of temperature was produced but no very

definite reaction, and on October 9th (being the fourteenth day after inoculation) it was bled and Goat 265 was inoculated subcutaneously with 100 c.c. of its blood and with 25 c.c. injected into the jugular vein.

On the following day there was a sudden elevation of temperature to 106·4°, and on the fifth day the animal died of characteristic heart-water.

The two previously inoculated animals remained meanwhile in seemingly good health.

Experiment 2: To prove that Goats Nos. 252 and 258 through which the virus had been transmitted while in a non-virulent form were in no degree protected thereby against subsequent inoculation with virulent virus.

Goat No. 266 was inoculated on October 10th with 100 c.c. of the blood of 265 by subcutaneous injection and with 20 c.c. injected intravenously.

On the 8th day the temperature ran up to 105·4° Fahr.

„	11th	„	„	„	104·8°	„
„	12th	„	„	„	106·4°	„
„	13th	„	„	„	104·4°	„

when it died of heart-water.

(*Note.* During the progress of these experiments clean goats were always kept with the experimental ones, and, at the close of the experiments they were all inoculated with virulent blood and all died of heart-water.)

Goat No. 252, which had been inoculated as already seen with non-virulent blood, was now inoculated on October 23rd with 30 c.c. of the blood of No. 266, by intravenous injection.

On the 6th day the temperature rose to 104·4° Fahr.

„	7th	„	„	„	107·6°	„
„	8th	„	„	„	107·4°	„
„	9th	„	„	„	106·6°	„

when it died of typical heart-water.

Goat No. 258, which also had been already inoculated 28 days previously with non-virulent blood, was now inoculated on October 23rd with 30 c.c. of the blood of No. 266 by intravenous injection.

On the 7th day the temperature rose to 107·4° Fahr.

| „ | 8th | „ | „ | „ | 103·6° | „ |

when the animal died of typical heart-water.

Goats Nos. 278 and 279 were each inoculated in the same manner as controls and in both cases the temperature began to rise on the eighth day afterward and death occurred on the eleventh.

In the above experiment, therefore, it is seen that the virus, which had originally passed through Goats Nos. 252 and 258 and was by that means raised to virulence, actually killed these animals when re-inoculated into them after its accession to virulence had been achieved.

Hence heart-water virus of which the virulence has been lowered does not necessarily afford protection to animals which have been inoculated with it.

Experiment 3: To prove, in such cases as those of animals Nos. 252 and 258, that an inoculation with weakened virus actually predisposes to subsequent infection with virulent blood.

In both of the above cases it is to be noticed that the incubation period was shortened as compared with the "control" animals, and I have further to add that this observation has been abundantly confirmed in a vast number of other cases.

Transmission of heart-water from goats to cattle.

Experiment 4: A clean ox from a sweet veld area was inoculated by the injection of 5 c.c. subcutaneously and 5 c.c. intravenously of blood from a goat which was dying of heart-water. On the 15th day after inoculation the temperature began to rise, reaching during the evening to 106·4°. During the five days subsequent it was maintained about 105° F. without remission, but the following morning it fell to 101·8° and on the same evening ascended to 107·2°. It died two days later.

On making a post-mortem examination I found the pericardium filled with fluid; there was some interlobular pulmonary infiltration and indeed there were produced conditions similar to those we are accustomed to find in goats dying of heart-water.

On the 16th day of the disease it was bled, and after defibrination of the blood, a goat was inoculated by the injection of 10 c.c. sub-cutaneously and 10 c.c. intravenously.

This goat died seventeen days later of typical heart-water.

The type of fever induced in cattle by the inoculation of horse-sickness blood is practically the same as that obtained by the inoculation of the same species of animal with heart-water blood.

The post-mortem appearances are likewise identical and agree in all particulars with those found in the endemic disease occurring in cattle

and known to the Kafirs as Imapunga, while having shown typical cases
to experienced transport riders they have assured me that it is known
to them as veld-sickness.

As I have already said, Karoo cattle coming to the coast areas
are liable to become attacked, the coast cattle remaining in perfect
health. Transport riders assure me that cattle from these coast areas
can travel throughout the whole of South Africa except in the Tsetse-
fly belts. I have ascertained that this disease occurs on the velds on
which heart-water is known to exist.

The co-relation of veld-sickness and heart-water.

Experiment 5 was made to determine the relation of Imapunga
or veld-sickness in cattle to heart-water in goats.

With blood taken from a Graaf Reinet calf dying of veld-sickness I
inoculated Goat No. 312 on Dec. 16th by the intravenous injection of
30 c.c. of the blood. The temperature began to rise on the 5th day
and the animal died on the 15th day of heart-water.

The post-mortem appearances were absolutely typical of heart-water
occurring spontaneously among goats.

Goat No. 327 was inoculated in the same manner with the blood of
Goat No. 312 on Dec. 30th and died on the 17th day of heart-water.

Goat No. 331 was inoculated in the same manner with the blood of
No. 327 and died on the 15th day with similar symptoms.

I could not detect the slightest difference between those cases and
cases of heart-water produced either spontaneously or by inoculation.

Experiment 6 : To show that goats born and reared on a farm
infected with veld-sickness are not so susceptible to that disease as
are goats which have been reared on a clean veld.

Goat No. 305 from a farm on which veld-sickness exists was
inoculated on Nov. 24th by intravenous injection of 20 c.c. of the
blood from a calf which died of veld-sickness. A slight reaction
followed immediately and soon subsided.

On Jan. 12th it received intravenously 30 c.c. of the blood of No. 327
at the same time as No. 331 of the previous experiment.

While this goat remained unaffected the clean goat No. 331 died.

Experiment 7 : To show that goats reared on a farm infested with
veld-sickness are relatively insusceptible but not immune.

Goat No. 315 from a veld-sickness infected farm, was inoculated

on December 14th with 10 c.c. injected subcutaneously and 10 c.c. intravenously of the blood of a calf which died of veld-sickness.

A slight febrile reaction of short duration followed. On the 12th January it received 30 c.c. intravenously of the blood of No. 327, and as a result died of the disease on the 13th day. (This result is in agreement with what we find obtaining among goats running on a heart-water veld when these are inoculated with heart-water.)

Experimental observation 8 : To show that goats reared and running on a farm infested with veldziekte are relatively insusceptible to heart-water.

In my prefatory remarks I alluded to the fact that goats purchased on the farm of Mr G. Palmer (which is a veld-sickness infested farm) were relatively insusceptible to heart-water but not immune, since although they very frequently resisted the intravenous injection of heart-water blood, yet if a second inoculation was made at a later date they commonly succumbed.

Experiment 9 : To prove that goats relatively insusceptible are not actually immune.

In almost every case where one of the goats from Mr Palmer's farm, inoculated with virulent blood either from Somerset station, Koonap, or that obtained by me from experimental goats, have withstood the intravenous injection of virulent blood I have found :—

(1) That they have been actually infected although showing no signs of disease, since with their blood I have been able to infect susceptible goats, which in some cases have died of the virulent malady.

(2) That if, after unsuccessful inoculation, they are allowed to remain in the Institute for several weeks, a subsequent intravenous inoculation of virulent blood is almost always successful in producing the disease and death.

Experimental observation 10 : To show that goats, on farms in this and adjoining areas, reared and living there, are relatively insusceptible.

I have already referred to the experiences of Messrs Hoole and White which suffices as evidence in this respect. Co-relation of horse-sickness to heart-water, to veld-sickness *in cattle and to a condition known as veld-sickness* in horses :—

If horses which have been reared in the Karoo are brought down to the coast areas it is usual to find that they fall off in condition, and in some cases die. From what I have heard and seen I am constrained to believe that this condition is that which was referred

to in the report of Lieut.-Colonel Joshua Nunn, F.R.C.V.S., A.V.D., to the Director-General of the Veterinary Department of H.M. War Office in 1888 as the biliary form of horse-sickness. Among the farmers it is, however, commonly referred to as veld-sickness.

In my communication to the Royal Society (Vol. 67) I referred to the results which I had obtained by the inoculation of donkeys with the blood of animals dying of horse-sickness and thereafter using the donkeys' blood for the inoculation of horses.

Since that communication was made I have been able to extend experiments of that class and the results may be summarized as follows:

1. The reaction produced in the donkey is no guide to the result which may follow the inoculation of its blood into a clean horse. The reaction may be slight or may be fatal.

2. If the donkey's blood is drawn at the tenth day and used to produce in a clean horse a violent reaction, then the blood of the same donkey if drawn two or three days later (without any further re-inoculation) will cause a much more violent reaction, and possibly death from virulent horse-sickness.

3. If a mild reaction is produced it may be of the nature of high temperature with remissions, or if still milder, may have a lower degree of fever with long intermissions.

In the case of animals which suffered from the last form of fever it was always noticed that they fell off in condition to a remarkable extent, becoming mere skeletons.

If killed or dying as late as the 50th day, one found evidence of horse-sickness in the form of exudation of serous material into the subcutaneous tissues, the interlobular tissue of the lungs, into the mesentery, the pleural cavity, and sometimes into the peritoneum. In the interventricular groove of the heart one always found some serous exudation, and the vessels lying here were always opaque owing to an infiltration into the vascular coats. The condition might be regarded as a sort of chronic horse-sickness.

The inoculation of 10 c.c. of heart-water blood into a clean horse produced similar phenomena, the animal dying two months after inoculation.

I have only made a few inoculations with heart-water into horses, and in some cases even a large dose (50 c.c.) has only produced a transient febrile reaction.

During the war, camps were formed for the receipt of farmers' horses, and reports reached me that horses running in some of these

camps were dying in large numbers from "poverty," "scab," etc., and as these camps are infested with veld-sickness it seemed to me that probably they were suffering from the "chronic form of horse-sickness" which I had experimentally produced.

On December 29th I proceeded to the protection camp at Thorn Park in this district accompanied by the local officers, Mr E. White and Mr Dalton. I saw no dead animals, as these had been already buried, and therefore decided to shoot any one which I might see in a poor condition. After several hundreds had galloped by I determined upon one which seemed poor enough, although it galloped quite freely. One of the officers then managed to bring it down with a rifle shot, and we at once proceeded to make a post-mortem examination, the appearances noted being as follows :—

The subcutaneous tissue was not invaded to any definite degree by exudation, although along the lines of the great vessels in the neck there was evidence that it had existed, but had coagulated and was now in process of absorption, leaving tough lines of dry exudation.—The pleural cavity contained about one gallon of a clear yellow serous fluid.— The lungs showed patches of congestion, some of which were deep liver-coloured. There was a definite amount of subpleural infiltration of serous fluid. There was also a widespread condition of interlobular infiltration of serous exudation.—The pericardium or heart-sac contained about 40 ounces of clear yellow or straw-coloured serous fluid, and some mass of coagulated gelatinous material produced by the coagulation of the fluid.—The base of the heart was surrounded by a huge gelatinous mass, and the interventricular groove was filled up by the same material.—The aorta and the larger vessels of the interventricular groove were invaded by the exudation, and the latter were rendered absolutely opaque, looking like white clay-pipe stems lying in a jelly.—Some fluid was also found in the peritoneal cavity, but no other characteristic pathological lesion was found.

Two days later Mr Dalton proceeded to another camp, and having shot a horse there, brought to me the heart and lungs *en masse*. The conditions found here were identical with the foregoing case, but rather more aggravated in type.

I cannot regard these and similar observations which I have made otherwise than as indicating that the condition which I produced in clean horses, by heart-water blood inoculation, and also by the injection of the blood of horse-sickness inoculated donkeys, is of the same nature as that which existed among the horses of the protection camps.

In cases where I carried my experimental inoculations so far as to produce perfect protection against horse-sickness, the animals immediately thereafter began to put on flesh.

Transmission of horse-sickness from horses to goats.

As I have already said the inoculation of even a large dose of heart-water blood into a horse may fail to be attended with any very definite result.

Conversely the inoculation of horse-sickness blood into goats was attended with uncertain results. In my first experiments, out of seventeen inoculated at different times, a febrile reaction occurred in only ten and none died. These goats, however, were obtained from Mr Palmer's farm. Since then I have used absolutely clean goats and have had further success.

On March 7th, 1902, I inoculated goat No. 381 with 20 c.c. of fresh horse-sickness blood by intravenous injection. It died three days later.

On post-mortem examination I found an enormous interlobular exudation into the lungs and pericardium. In the latter the whole exudation was absolutely solid.

This remarkable result is somewhat to be compared with experiments which I made a few years ago in inoculating a goat and a sheep with the serum of a " salted " goat which had been reinfected by inoculation a week previously.

The sheep and goat were inoculated in the forenoon and were found dead the next morning with symptoms very similar to those just recorded.

With the blood of goat No. 381 I now inoculated No. 383 by intravenous injection of 5 c.c. of defibrinated blood on March 9th.

Some fever followed, and on the 10th day it had a temperature of 106°, making however a good recovery.

On March 20th I bled this goat and inoculated No. 393 with 5 c.c. by intravenous injection.

After an incubation period of six days the temperature began to rise and the animal died on the 16th day. On making a post-mortem examination I found the usual signs of heart-water.

No. 393 was used to inoculate No. 408, which died on the 14th day.
No. 408 was used to inoculate No. 411, which died on the 13th day.
No. 411 was used to inoculate No. 419, which died on the 11th day.

This experiment which has been carried out with every care as regards the keeping of control animals in contact during the experiment and subsequently showing by inoculation that the controls were still susceptible to virulent infection, admits me to say that horse-sickness can be transferred to goats, and that, when acclimatized to the goat, it produces in this animal a virulent disease which is indistinguishable from the endemic disease of goats which is known in South Africa as heart-water.

Heart-water produced by the inoculation of the blood of *protection-camp horses*:

I was enabled to get a protection-camp horse sent into the Institute and while there I bled it and inoculated goat No. 365 with 30 c.c. intravenously and 30 c.c. subcutaneously on February 11th.

On the 7th day the temperature rose and remained high, 105° F. and slightly over, until the 11th day, when it fell to 101° and the animal then died.

On making a post-mortem examination I found oedema of a semi-transparent character at the base of the heart extending up the aorta. The lungs were pale, but the left lung had a dark patch of congestion about two inches in diameter, and being sharply circumscribed within a group of lobules. The pericardium was quite filled with a clear yellow serous fluid which quickly coagulated when transferred to a glass. The conditions found were thus absolutely typical of what one obtains in ordinary heart-water.

I therefore conclude that the contagium which causes horse-sickness in the equids of South Africa is responsible, under conditions of relative virulence, for the infection of other species of the domesticated animals.

The means by which the virulence becomes relatively altered is not entirely clear, but my colleague the Colonial Entomologist has been able to produce heart-water in goats and calves at Cape Town by means of the progeny of bont-ticks taken in the Eastern Province from infected goats. In this way therefore the very striking observation of the late Mr Webb has been proved to be correct. One is not, however, yet able to say that heart-water is not conveyed by any means other than the bont-tick.

THE COLON BACILLUS IN GROUND WATERS[1].

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(From the Laboratory of the Ohio State Board of Health.)

During the past four years it has been part of our routine work to examine samples of ground waters from wells and springs in various portions of Ohio, and, in so doing, organisms have been obtained from time to time conforming to the tests usually given as differentiating faecal bacteria.

The mere finding of *B. coli* in ground waters is not new, as its presence in several cases has been mentioned by other workers[2,3], but we desire especially to call attention to the significance of the presence of *B. coli* in ground waters, *i.e.* ground waters derived from springs or drilled wells.

Naturally there have been many samples from dug wells in the waters of which the colon bacillus was present, but in the present article we omit all reference to such samples, and include only those derived from springs and drilled or bored wells that at the same time contained *B. coli*. The list includes 25 samples from wells varying in depth from 16 to 243 feet, although most of the wells were from 25 to 80 feet deep. Almost without exception these wells were cased, and with the casing extending above the level of the ground, thus preventing the direct entrance of surface water. The list also shows 12 samples of water from springs in which the colon bacillus was found.

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[1] Read at the meeting of the Laboratory Section of the American Public Health Association, New Orleans, La., Dec. 8, 1902.

[2] Weissenfeld, *Zeitschr. f. Hygiene*, Vol. xxxv. p. 78.

[3] Savage, *Journ. of Hygiene*, Vol. ii. p. 320.

Part of the springs were open to surface pollution, while others appear to have been well protected.

With a few of the samples the analysis was bacteriological only, but generally there was also a more or less complete sanitary chemical examination.   The number of bacteria per cubic centimetre varied from 16 to 900,000 in the well waters, and from 90 to 400,000 in the samples from the springs.   Some of the chemical and bacterial analyses gave marked evidence of sewage pollution, indicating a somewhat open course from some sewage source to the water-producing strata in question.   In other cases the chemical evidence of pollution was small, while in still others there was no chemical evidence of sewage pollution, and the waters would have been passed without question had there been only a chemical analysis.

Since some of the waters under examination were those proposed as public supplies for villages or cities, and some were from localities where typhoid fever was or had been present, we did not feel warranted, at the present time, in accepting as final any of the gross, or preliminary, or presumptive tests, although such were made use of in connection with the more laborious isolation of species and identification by cultivation on various media, etc.   The final conclusion depended almost entirely on the results obtained by the latter process.

During the period of this study different methods have been resorted to for the isolation, and much of the time two or more methods were employed simultaneously.   The methods have been as follows:

(*a*)   Preliminary cultivation in Dunham's peptone, using 40 or 90 c.c. of the water in question, and followed by plating to lactose-litmus-agar (or in a few cases to gelatin) from which colonies were fished and subcultivations made.

(*b*)   Cultivation in glucose broth, using 40 or 90 c.c. of water with subsequent plating, etc.

(*c*)   Same as (*b*) except that 1 c.c. of the water was used.

(*d*)   Cultivation in carbolated sugar broth, using 40 or 90 c.c. of water with subsequent plating, etc.

(*e*)   Same as (*d*) except that 1 c.c. of water was used.

After plating, it was customary to fish from four to six colonies and test as follows: morphology, motility, litmus milk, nitrate solution, peptone for indol, neutral-red, gelatin stab, gas production in sugar broth, gas formula of 2 : 1.   A portion of the above individual tests were omitted with some of the samples, but not with the more particular ones.

In view of the work by Prescott[1] on *B. coli* and allied forms, the question arises as to whether the organisms isolated were colon bacilli, and, accordingly, other than bacteriological evidence was sought in the history of these waters in relation to typhoid fever among the users. Of the 37 samples, 27 gave a history of typhoid fever, the number of cases per well ranging from 1 to 16, and from 1 to 17 for the springs. Of the remaining samples two were from springs that could hardly escape animal pollution. With the evidence at hand we feel warranted in saying that the organisms isolated were colon bacilli, and that the presence of this organism is at present to be looked upon in spring waters with grave suspicion, unless there is known opportunity for surface pollution carrying waste material from the lower animals. The results obtained lead us to believe that the presence of *B. coli* in waters from drilled wells ought in most cases to condemn the use of that water for domestic purposes.

An exceptional case may be stated here. A drilled well 35 feet in depth was put down for the proposed supply of a village near the central portion of the State. On examining the water the more important chemical findings were as follows in parts per million: oxygen consumed, ·46; N as albuminoid ammonia, ·039; N as free ammonia, ·014; nitrites, none; nitrates, trace; chlorine, 33·1. (It should be stated that the chlorine is not a close indicator in some parts of Ohio owing to the presence of salt or salt waters.) The number of bacteria per c.c. was 18. In the qualitative bacterial examination, each of the above individual tests was applied to the organisms isolated with positive results, *i.e. B. coli* was present. A second sample a month later gave the same organism, but in view of the excellent chemical analysis, the low number of bacteria, and the unqualified recommendation of the Civil Engineer of the State Board of Health, who had made a personal inspection of the locality, the sample was allowed to pass. Subsequently an examination was desired, but unfortunately the portion for bacterial examination was not taken by the collector, and only a chemical analysis was made. This analysis showed even better results than the former one.

[1] Prescott, *Abstract in Science*, N.S. xv. p. 363 (1902). (Also a paper read at New Orleans, La., American Public Health Association Meeting, Dec. 8, 1902.)

Conclusions.

1.   We believe the organism found in these waters from springs and drilled wells was *B. coli*.

2.   The presence of *B. coli* in spring water should be looked upon with suspicion, unless there is apparent opportunity for pollution by the lower animals.

3.   The presence of *B. coli* in water from a drilled well should generally condemn that water for domestic use.

4.   The fact that a water is derived from a drilled well should not be taken as an absolute guarantee that it is a potable water.

5.   Both chemical and bacterial examinations of a water are desirable, but the bacterial will sometimes prove the more delicate indicator of direct pollution, which is the more dangerous pollution.

SOME EXPERIMENTS ON THE INTRAVASCULAR USE OF ANTISEPTICS.

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THE prominence given to the intravascular use of antiseptics has hardly been in accord with the results achieved by this method.

The injection of formalin in various amounts and strengths has been given an extended trial in the treatment of pulmonary tuberculosis, and is claimed to have produced beneficial results[1]. Similarly Baccelli has injected corrosive sublimate intravenously in the treatment of syphilis, cerebro-spinal meningitis, and acute rheumatism in man. In animals he has used intravenous injections of corrosive sublimate in the treatment of aphtha epizöotica. In this disease he claims that the intravenous injections of corrosive sublimate are of undoubted use in terminating an attack of the disease[2].

Further, Baccelli states, "It is finally a hygienic measure, preservative, as it destroys *in situ* the source of infection[3]." That is, he claims that antiseptics act as such when injected intravenously. But in the above instances other methods of treatment have been simultaneously applied and the cure, if any, might not have been due to the antiseptic part of the treatment.

Ewart has reported favourably on the intravascular injection of protargol in cases of tuberculosis; and Behring, in 1887, thought that silver oxide had a favourable effect on the course of experimentally produced anthrax in animals. Cash has also obtained positive results with corrosive sublimate[4].

[1] *Proc. Brit. Congr. Tuberculosis*, 1901, Vol. III. p. 438.
[2] *Lancet*, Jan. 3, 1903.   [3] *Boll. Chem. Farmaceut.* Feb. 1902.
[4] *L. G. B. Med. Off. Reports*, 1884 and 1885.

Against these we have the evidence of Washbourn[1], who obtained negative results with creolin; of Koch and Behring[2], who obtained negative results with corrosive sublimate; and of Serafini, who also obtained results unfavourable to the intravascular use of antiseptics. Further, the experiments of Fortescue Brickdale[3] are against the utility of the intravascular injection of antiseptics, though in his experiments only small quantities of antiseptics were pitted against the highly resistant *B. anthracis, e.g.* only 1 c.c. of 1 % formalin.

The experiments recorded in this paper were undertaken for the purpose of investigating the value of antiseptic injections into the circulation. The antiseptics employed were formalin, guaiacol, and chinosol. The following experiments were first made with the object of ascertaining how much of these substances could safely be injected. The point of chief importance was to determine the maximum non-toxic doses in each case, as it has been shown that tolerance of these drugs cannot be increased by repeated injections.

Healthy full grown rabbits were the animals used in these experiments, and in each case the injections were made into the marginal vein of the ear, with a sterile needle and syringe, and most careful precautions were taken against air embolism. The results are recorded in the following table (Table I.).

TABLE I.

	Rabbit D.	Rabbit F.	Rabbit G.	Rabbit H.
	Injected with 8 c.c. Chinosol 1/640 in 0·6 % NaCl solution	Injected with 25 c.c. Guaiacol 1/200 in 0·6 % NaCl solution	Injected with 10 c.c. Formalin 1/250 in 0·6 % NaCl solution	Injected with 15 c.c. Formalin 1/500 in 0·6 % NaCl solution
1902 June 2	2500 grms. (8 c.c.)	2520 grms. (25 c.c.)	2460 grms. (10 c.c.)	1510 grms. (15 c.c.)
,, 3	2550 grms.	2510 grms.	2430 grms.	1500 grms.
,, 4	2480 ,,	2530 ,,	2450 ,,	1520 ,,
,, 5	16 c.c. of 1/640 chinosol intravenously 2540 grms.	2520 ,,	2480 ,,	1525 ,,
,, 6	2520 ,,	2545 ,,	2470 ,,	1520 ,,
,, 7	2570 ,,	All well and active, no paralysis.		
,, 8	—			
,, 9	16 c.c. chinosol 2550 grms.			
,, 10	2650 ,,			
,, 11	2600 ,,			
,, 12	2660 ,,	(Total 40 c.c. 1/640 chinosol.)		

[1] *Guy's Hosp. Reports*, 1888.
[2] Behring (1894) *Bekämpfung der Infektionskrankheiten*, p. 35.
[3] *Lancet*, Jan. 10, 1903.

The point next considered was the organism against which the power of these antiseptics should be pitted. The intravenous inoculation of anthrax bacilli was found to produce uncertain results; the duration of the illness and the effect produced depend so much upon the virulence of the particular culture used, and even more so on the amount of emulsion injected. Moreover, *B. anthracis* is a highly resistant organism when tested against antiseptics *in vitro*; and for the purpose of these experiments a more susceptible organism would give a better indication of the value of antiseptic injections.

For this reason experiments were made with the *B. coli communis*, and *B. typhosus*. But the particular cultures used were unsatisfactory. They either caused death in 5 or 6 hours, or produced a lingering illness from which the animals often recovered. Equally unsatisfactory were the results of intravenous inoculation with *Streptococci* and *Pneumococci*. The animals died so quickly that little opportunity was given to investigate their condition.

I. *Experiments with Rabbits infected with B. pyocyaneus.*

The *B. pyocyaneus* was found to be the most useful organism, producing uniform and concordant results when inoculated intravenously. Several experiments were undertaken to determine the pathogenic power of this organism. Cultures were made on standardized glycerine-agar, and incubated for 15 hours previous to inoculation. An emulsion was made of the whole of one such agar culture with 5 c.c. of sterile normal saline solution.

Using this emulsion of *B. pyocyaneus* for inoculation a series of eight experiments gave the following results:

1 c.c. killed rabbits of 1480—2100 grms. in 3—5 days.
2 c.c.   ,,   ,,   1530—2210   ,,   1—3   ,,
3 c.c.   ,,   ,,   1560—2460   ,,   12—15 hours.
4 c.c.   ,,   ,,   1460—2540   ,,   5—8   ,,

These results were confirmed from time to time by the control injections made in the course of the following experiments on the efficacy of antiseptics injected intravenously during the course of the disease.

The experiments will be given *in extenso* so that the effect of the antiseptic may be noted in each case.

(1) *Guaiacol solution*, 1/200.

Rabbit 1. 2530 grms.

Injected with 2 c.c. emulsion of *B. pyo-cyaneus* and immediately afterwards with 20 c.c. of guaiacol solution.

Died in 18 hours.

Rabbit 2. 2510 grms. (Control).

Injected with 2 c.c. of emulsion of *B. pyo-cyaneus*. No antiseptics injected.

Died in 26 hours.

B. pyocyaneus recovered at autopsy from the blood of heart in both rabbits.

(2) *Chinosol solution*, 1/640 of 0·6 % NaCl.

Rabbit 1. 1780 grms.

Injected with 2 c.c. of emulsion of *B. pyo-cyaneus* and immediately afterwards with 15 c.c. of chinosol solution.

Died in 17 hours.

Rabbit 2. 1820 grms. (Control).

Injected with 2 c.c. of emulsion of *B. pyo-cyaneus*. No antiseptics injected.

Died in 20 hours.

B. pyocyaneus recovered at autopsy from blood of heart of both rabbits.

(3) *Formalin solution*, 1/500.

Rabbit 1. 1860 grms.

Injected with 2 c.c. of emulsion of *B. pyo-cyaneus* and immediately afterwards with 15 c.c. formalin solution.

Died in 20 hours.

Rabbit 2. 1920 grms. (Control).

Injected with 2 c.c. of emulsion of *B. pyo-cyaneus* but no antiseptics intravenously.

Died in 23 hours.

B. pyocyaneus recovered at autopsy from the heart blood in the case of both rabbits.

(4) *Formalin solution*, 1/250.

Rabbit 1. 1940 grms.

Injected with 2 c.c. of emulsion of *B. pyo-cyaneus* and immediately afterwards with 18 c.c. of formalin solution.

Died in 5 hours.

Rabbit 2. 2100 grms. (Control).

Injected with 2 c.c. of emulsion of *B. pyo-cyaneus*.

Died in 24 hours.

B. pyocyaneus was recovered from the blood of the heart of both rabbits at autopsy.

The rabbits in experiments 3 and 4, injected with formalin, began to sneeze before the injection was completed. A watery discharge of nasal mucus soon began, and the rabbit frequently brushed its nose with its paws.

In each of the above experiments the injection was made into the marginal vein of the ear. The ear was previously cleaned and disinfected by scrubbing with iodide of mercury solution of a strength of 1/1000.

As a control to the above intravascular injections of *B. pyocyaneus* followed by antiseptics, experiments were made as to the efficacy of formalin incubated with broth cultures of the bacillus. Broth cultures were mixed with varying quantities of formalin and incubated for 15 hours as follows:

B. pyocyaneus in 1/1000 Formalin broth—no growth.

  ,,   ,,   1/1500   ,,   ,,   —no growth.

  ,,   ,,   1/2000   ,,   ,,   —growth completely arrested.

These experiments show that this bacillus is susceptible to the action of formalin. If, then, the solution of formalin when injected intravenously, acted directly as an intravascular antiseptic, the amounts used in the animal experiments were sufficient to give positive results. But in all cases the injected animals died more quickly than the control animals, and *B. pyocyaneus* was recovered from the blood of the heart in every case.

These experiments show conclusively that the course of a septicaemia, such as that produced by *B. pyocyaneus*, is not checked but rather accelerated by the intravenous injection of the antiseptics used. Moreover, where, as in experiment 4, with 1/250 formalin, a very large dose, 18 c.c. was injected, the growth of the bacilli was not arrested. The formalin was probably rapidly taken up by the tissues. If it had remained in the blood, the amount present would have been amply sufficient, as shown by the control experiments with formalin-broth, to completely arrest the growth of the bacilli. The animal used would have less than 100 c.c. of blood, so that when the blood mixed with the injected formalin the latter would still be present in a strength of about 1/1500, if no formalin escaped from the blood vessels.

From results such as these the value of intravenous injections of antiseptics would seem of but little use in checking the course of the disease produced by the action of *B. pyocyaneus*. And from this it is but fair to infer that the clinical use of intravenous injections of *antiseptics* can have but the very smallest amount of therapeutic value in cases of septicaemia.

II. *Experiments on Tuberculous Rabbits.*

To investigate the action of formalin in the course of a more chronic disease than the preceding, the following experiments with tuberculous rabbits were undertaken. Rabbits are less susceptible to tuberculosis than guinea-pigs, and hence give a more favourable opportunity for observing the effects of the antiseptics.

In order to produce a definite infection of the lungs, and thus reproduce, in some measure, one of the forms of clinical tuberculosis, the rabbits were infected intravenously, the usual method of peritoneal inoculation not being found suitable.

Some guinea-pigs had previously been infected by subcutaneous inoculations with human tuberculous sputa. From one of these guinea-pigs, after being killed with chloroform, a tuberculous gland was

removed and made into an emulsion with sterile normal saline. Of this emulsion, two equal portions were injected intravenously into two (*control*) rabbits *A* and *B*. Rabbit *A* was soon noticed to be ill and losing weight. It died 9 days after injection, and was found to have been suffering from tuberculous pericarditis, pleurisy, and pneumonia. Rabbit *B* was not obviously affected for some time; but died 27 days after injection, of tuberculous pneumonia.

Two other rabbits, *C* and *D*, were then infected with tuberculosis. Each was injected with 4 c.c. of an emulsion of a guinea-pig's tuberculous gland as before. The immediate effect of the inoculation was *nil*. But in ten days both rabbits had lost weight:

> Rabbit *C* weighed 2520 grms., now 2140 grms.
> „ *D* „ 2350 „ „ 2180 „

Formalin injections were now given to the two rabbits to ascertain their effect on the course of the tuberculosis.

Rabbit C received 10 c.c. of a 1/500 solution of formalin in normal saline solution intravenously. This caused it to sneeze, and a watery discharge of nasal mucus to run from its nose. The breathing soon became very rapid: 140—150 respirations per minute. In 20 minutes the rabbit was very ill, lying upon its side, and it died in less than 30 minutes from the time of the injection of formalin.

The experiments above recorded, though few in number, appear to indicate that the lethal effect, in this case, was not alone due to the amount of formalin injected (see the experiment on a healthy animal, p. 160). The tuberculosis had made the animal less resistant to the injection of formalin.

The examination of this rabbit's viscera at autopsy showed extensive caseating tubercles throughout the lungs, a fatty and enlarged liver, and a spleen studded with tubercles. Microscopically the lungs were found to be in a condition of acute miliary tuberculosis, due to the *B. tuberculosis*. It was this condition, which, impeding the blood-flow through the lungs, had prevented the rapid passage of the formalin through the pulmonary capillaries. The danger of injecting formalin in this condition is thus very great, as the above experiment shows. Apparently the formalin concentrates its effect on the lungs and hinders their normal functional activity.

Rabbit D also received an injection of 10 c.c. of a solution of 1/500 formalin in normal saline solution. The injection produced no

immediate effect, and the rabbit did not appear to be quite as ill as rabbit *C*.

The injections were repeated as follows :

Rabbit D.

				Weight of rabbit
10th day after inoculation—10 c.c. of 1/500 formalin				2180 grms.
14th ,,	,,	10 c.c.	,, ,,	1950 ,,
17th ,,	,,	10 c.c.	,, ,,	1750 ,,
21st ,,	,,	5 c.c.	,, ,,	1680 ,,
24th ,,	,,	Rabbit died		1630 ,,

The autopsy on rabbit *D* showed that the lungs and spleen contained numerous tubercles. Microscopically these tubercles contained tubercle bacilli in large numbers.

Rabbits infected, therefore, in this manner with tuberculosis may be expected to die in about a month, or under exceptional circumstances, as in rabbit *A*, in 9 days. The injections of formalin in rabbit *D* would not seem to have affected the course of the disease favourably or otherwise. In the case of rabbit *C* the dangers of formalin injection are demonstrated. The obstruction offered by the miliary tubercles to the flow of the formalin through the lungs, concentrates its effect there, and this, though desirable therapeutically, appears to be disastrous physiologically.

The results of these experiments would seem to show that there are no advantages to be derived from the intravascular use of antiseptics.

STUDIES IN RELATION TO MALARIA.

II (concluded).

THE STRUCTURE AND BIOLOGY OF ANOPHELES

(*Anopheles maculipennis* Meigen.)

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THE DIGESTIVE ORGANS.

THE alimentary canal of *Anopheles* consists of the following parts:—
1. Mouth, 2. Buccal cavity, 3. Pharynx or Pumping-Organ, 4. Oesophagus, with which are connected, 5. Three food reservoirs (two situated dorsally and one ventrally), 6. Oesophageal Valve, 7. Mid-gut, 8. Ileum, 9. Colon, 10. Rectum, 11. Anus. (See Plate VI, Fig. 1.)

Judging by analogy with other insects, the chitinous lining of the parts numbered 1—5 (mouth to oesophagus inclusive) indicates that they arise from the embryonal stomodaeum. The mid-gut has its origin from the mesenteron. The termination of the mid-gut and commencement of the colon correspond with the point where the Malpighian tubes are connected with the alimentary canal. The chitinous lining of these tubes indicates their origin from the proctodaeum or hind-gut. The alimentary canal lies above the ventral nerve-cord and ganglia and beneath the heart or dorsal vessel.

The salivary glands do not communicate with the alimentary canal.

The examination of a series of sections of the proboscis of a female *Anopheles maculipennis*, passing from the tip to the base, shows that the various parts—maxillae, mandibles, labrum-epipharynx, hypopharynx, labium, even the maxillary palps—have their origin at about the same level. That level is a little way behind the anterior limit of the clypeus, and a section through this region shows all these parts closely adpressed one against the other but still discrete. A section or two further back shows that these parts become fused with one another and that they surround the beginning of the alimentary canal.

The Mouth and Buccal Cavity.

(See Plate VII, Fig. 5.)

The region where the mouth-parts coalesce, as described above, we style the *mouth*[1]. The *buccal cavity* extends from the mouth to the valvular arrangement situated at its juncture with the pharynx. The general direction of the buccal cavity is upward and backward; the portion which approaches the pharyngeal orifice is directed more suddenly upward. Close to the mouth the lumen of the buccal cavity resembles that of a railway tunnel, measuring (in the female) but 33μ across its floor and 25μ in height, it then becomes complicated by the origin of two wing-like chitinous processes which project upward and serve as apodemes for the insertion of muscles. The lumen next narrows to a horizontal slit in the middle, but pointing upward and outward at the sides. This change of form is due to the stout chitinous floor projecting upward at the sides. Passing beneath the floor is the well-developed paired muscle running forward to the salivary "pump" and having its origin from a chitinous flange which is continuous with the chitinous floor of the buccal cavity. Towards its posterior end the lumen of the

[1] It will be noticed that we divide the fore-gut into more parts than is usual, this being merely a matter of convenience. In insects the alimentary canal may be divided into three main divisions, in accordance with their embryonic origin, viz. fore-gut, mid-gut, and hind-gut; derived respectively from the stomodaeum, mesenteron, and proctodaeum. The fore-gut, in a typical insect, is usually divided into regions known as the mouth, pharynx (which includes our buccal cavity), oesophagus, crop (ingluvies), and proventriculus (gizzard). The hind-gut, where more highly developed than in *Anopheles*, has been divided into ileum (short intestine), long intestine, colon, and rectum.

Dimmock styles our buccal cavity "pharynx," and includes our pharynx with the oesophagus. Annett and Dutton describe our buccal cavity as the "ascending portion of the pharynx." The structure of the parts we have described separately as buccal cavity, pharynx, and oesophagus, with their sharp limitations, lend themselves best we think to the divisions we have given, our terminology having the advantage of terseness.

buccal cavity again becomes circular in section, and it is at this point that the flange just referred to projects from the floor, the flange bending forward and the paired muscle being attached to its concave surface. Whereas the floor of the buccal cavity is lined throughout with stout chitin, its roof is less chitinous. Beginning again with the mouth, Annett and Dutton (1901, p. 84) describe and figure low papillae, which they term " taste-papillae " projecting from the chitinous roof, or palate as we shall term it, and apparently consisting of chitin, judging from their figure. They appear to represent blunted chitinous protuberances, and we do not know on what evidence the authors named attribute a sensory function to these minute structures, which they found in *Anopheles costalis*.

In *Anopheles maculipennis* we have noted the existence of an interesting structure in this region. The anterior portion of the palate is lined with thick chitin, this being followed by a " soft palate," (Fig. 5, Plate VII) lined with a distinct membrane, on the wrinkled surface of which there is a delicate layer of chitin, such as is demonstrable in the oesophagus by maceration in caustic potash. Posterior to the soft palate, thick chitin again makes an abrupt appearance, and this is continuous back to the pharyngeal valve. Anteriorly, the thickened chitin of the palate projects backward somewhat in the form of a trowel, the convexity being directed ventrally. The end of the trowel is continuous with the lining of the soft palate. In longitudinal section, the trowel is seen to end in a series of blunt projections apparently due to transversely folded chitin, the structure being very minute, and only clearly visible by the aid of a $\frac{1}{12}$th immersion. These correspond to the so-called " taste-papillae " of Annett and Dutton. Here the soft palate begins. Anterior to the protuberances just mentioned are situated short and minute spines of chitin, apparently about six in number and directed slightly forwards. The spines are hollow and are moveable, being articulated upon or within a ring of thickened chitin which is continuous with that of the palate. These spines appear to be in direct communication with strands of the anterior pair of the ten palatal muscles. In the chitinous floor of the mouth, directly opposite, a depression (seen in cross section) is noticeable which has a corresponding form to that of the structure just described, so that it appears that the lumen of the buccal cavity in this region may at times be almost occluded. Transverse sections midway through the soft palate moreover show two similar spines protruding, some distance apart, from

discrete annular thickenings of chitin, the spines being directed downward into the lumen of the buccal cavity. The significance of these structures is not clear, possibly they act as teeth or as strainers of particles of food ingested.

The wrinkled surface of the membrane lining the soft palate indicates that the lumen of the cavity may be considerably increased at times, this being clearly effected by the palatal muscles attached to the dorsal surface of the palate and running upward to their origin from the inner surface of the roof of the clypeus. What we have termed the palatal muscles are those which Annett and Dutton call the "oblique central fibres of the pharyngeal muscles." These authors do not figure the very marked membrane of the soft palate, stating that it is lined by flattened epithelium only and no chitin.

The thin layer of chitin lining the palate renders it easy, we consider, for the palatal muscles to draw the palate upward, and away from the floor. These muscles are five in number and paired. The structure we describe indicates that the buccal portion of the alimentary canal may well exert a suctorial action.

The valvular arrangement at the entrance to the pharynx was first described by Dimmock (1881, p. 13), who thought it "a valve to prevent the return of fluids to the mouth during the pumping process." This valve lies just behind the posterior end of the clypeus, the buccal and pharyngeal cavities uniting at an angle with regard to their axis at this point. There is evidence that the clypeus may be slightly moveable dorso-ventrally upon the head, and consequently the angle at which these cavities join may vary somewhat during life.

In this region the latero-dorsal chitinous wings are conspicuous, the chitinous structure being kept in position by two paired muscles. Two of these pass close to one another in the middle line of the vertex, outwards to each chitinous wing. The other pair of muscles arise each from the under surface of the chitinous wing, and, after a short course, are inserted into the tubular struts which pierce the head (see *Journal of Hygiene*, Vol. I. p. 482, Plate IX, Fig. 13, *tu*). Immediately in front of the two fine muscles running to an apodeme in the dorsal middle line, and the anterior line formed by their insertion into the dorsal surface of the buccal tube, lies the buccal ganglion. Close behind these two muscles are a second pair which run down parallel to one another from the middle of the vertex to be inserted into the anterior part of the pharynx.

In *Anopheles costalis*, according to Annett and Dutton (p. 85 and

Plate XVIII), at the junction of buccal cavity and pharynx, and attached at the angle of junction, ventrally, there is "a peculiar ridge of chitinous stout hair-like processes, which curve forwards so that their tips lie in the angle formed between the upper parts of the first (our buccal cavity) and second parts (our pharynx) of the pharynx. The hairs are of two kinds, an anterior large set—probably a single row—and a posterior, small, fine set situated in a clump immediately behind the former. The larger hairs consist of a short stout shaft firmly embedded in the chitinous pharyngeal wall; this shaft supports a cup with a free rim curved outwards; within the cup lies the oval-shaped bulbous extremity of the base of the hair; this bulbous extremity contains a single large cell. The remaining free portion of the hair curves forwards and tapers to a fine point, and appears to have a central shaft enclosed within a chitinous cuticle from which barb-like processes project. The hairs of the posterior set are much finer and shorter, and are much more numerous; they appear to be simple in character. In transverse section this structure presents to some extent the appearance of 'rods and cones.' The suboesophageal ganglion lies in close proximity to this structure, but no nerve-fibres have been traced to communicate with these specialised hairs, although such probably exist." These hairs appear to be sensory, and moreover they should aid in rendering the valve more effective, for the reason that they project into a space formed by the chitinous portions opposite. We have not as yet observed this interesting structure in *A. maculipennis*, and propose, when we have more material, to study the structure of the valve more closely.

The Pharynx or Pumping Organ[1].

(See Plates VI, VII, and VIII.)

The pharynx extends from the valve just described, to near the back of the head, where it joins the oesophagus. The anterior portion is

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[1] Packard (p. 303) states that Meinert (" Trophi Dipterorum ") described the pharynx as the principal, and in most Diptera as the only part of the pumping apparatus (antlia). Meinert appears to have recognised the nature of the mechanism. His figure of the pump, given by Packard (p. 78, figure 81) and the muscles attached thereto, the "musculis antliac," taken from *Culex pipiens*, is fairly accurate. A similar apparatus is also present in other flies, in Hemiptera and Lepidoptera. Dimmock (1881, p. 19) rightly states: " This bulb is the chief sucking organ in the female *Culex* "; he is in error however when he states (p. 13) that it is absent in the male insect.

tubular, and is strengthened by longitudinal chitinous ridges (Plate VII, Fig. 1). This part shows a dorsal flexure, which is better marked in the male insect (Plate IX, Fig. 2). The chitin with which it is lined throughout is continuous with that of the buccal cavity, and is seen to consist of a dorsal and two latero-ventral plates, which widen and again narrow posteriorly. Separated from one another, by dissection, or crushing beneath the coverglass, the three chitinous plates have somewhat the form of a narrow ping-pong racket, the distal or broadened end adjoining the oesophagus, showing rodlike sinuous chitinous ridges, the chitin ending in very fine spines, giving it a somewhat comb-like appearance (Plate IX, Figs. 1 and 2); cross sections in this region show that the chitinous rods project backward and slightly into the lumen of the commencement of the oesophagus, where they doubtless act like combs in straining out particular matter. This region is surrounded by a sphincter muscle. This structure has not been as yet observed (see Plate VII, Fig. 4). The study of the structure of the pharynx is greatly facilitated by the use of caustic potash, the soft parts being removed. Instructive specimens may be prepared, showing its relation to the other chitinous portions of the head, by macerating the heads of the insects, and afterwards cutting away the eye or other parts so as to expose the pharynx *in situ*. In this way also the pharynx may be dissected away from the rest of the head together with the appendages (Plate IX, Figs. 1 and 2). Viewed in cross section through the enlarged portion the three plates are seen to be connected together at their edges, which show delicate serrations running to the margin, and are curled outward. The junction of the plates is effected by means of a thin grooved gutter of chitin (V-shaped in section) in such a way as to permit of the expansion of the lumen by the muscles which are attached to and draw the expanded plates asunder. As will be seen by reference to Figs. 2 and 3 (Plate VII) the chitinous plates are curved, so that when the divaricating muscles are at rest the plates by virtue of their elasticity narrow the triradiate lumen of the pharyngeal bulb.

The pharynx is considerably larger in the female than in the male insect. In the male the tubular portion is relatively longer, the whole length of the pharynx is but 4/5 that of the female's. The width of the plates which compose the bulbous portion is about twice as great in the female as in the male, and the plates are composed of much thicker chitin, as will be seen by reference to Figs. 1 and 2, Plate IX.

We add some actual (averaged) measurements in round numbers, made on specimens treated with caustic potash:

> Length of pharynx in two males = ·32 mm.
> Width of widest portion of plates = ·09 mm.
> Length of pharynx in three females = ·40 mm.
> Width of widest portion of plates = ·20 mm.

The pharynx is obviously the chief organ by means of which the mosquito sucks up fluid food or blood. The most conspicuous and powerful muscles in the head are those whose function it is to draw apart the three walls of the bulbous portion of the sucking pharynx (Plate VII, Figs. 2, 3 and 5). Two, the posterior dorsal dilators, run side by side, straight downward from the occiput behind the brain and eyes to the median dorsal plate. Two others, the anterior dilators (or in the female two pairs), run in front of the brain from the vertex to the pharynx. The latero-ventral dilators consist of five distinct muscles on each side which run from the lateral posterior angle of the head inwards, upwards, and a little forwards to be inserted in the latero-ventral plates. When these muscles contract simultaneously, the almost slit-like triradiate lumen of the pharynx must become nearly circular in cross section, and suction will take place through the epipharynx, so that if the insect has its proboscis fixed in any animal, plant, or fluid—blood, sap, or the fluid will be drawn in. When the muscles relax, the food will be propelled backward into the oesophagus by the elasticity of the released plates.

The lumen of the end of the pharynx is triangular, that of the oesophagus which follows is rounded. The anterior tubular portion of the pharynx passes through the ring formed by supra- and infra-oesophageal ganglia and their commissures.

The Oesophagus.

The oesophagus is short, and extends from the posterior end of the chitinous pharynx to the oesophageal valve. Anteriorly, where it traverses the insect's neck, it is narrow, it then gradually widens, hardly to the extent that it can be called a crop. The oesophagus is plentifully supplied with bands of muscles, the posterior part being thrown into transverse folds when the circular fibres are contracted (as is indicated in Plate VI, Fig. 7). The oesophagus may be seen to undergo vigorous rhythmical contractions for hours when dissected out from the body and

placed in salt solution. In hardened sections it at times offers a spacious lumen, at others it is all but occluded. The shape of the epithelial cells lining the tube varies according to the degree of contraction or expansion. Their average outline is roughly cubical, their nuclei being sharply defined and slightly oval. The general structure corresponds in detail with that of the mid-gut to be considered presently.

The Food Reservoirs.

(Plate VI, Figs. 1, 4, and 7.)

The posterior end of the oesophagus lies on about a level with the origin of the first pair of legs, as pointed out by Grassi, and here are given off the three food reservoirs. The large ventral reservoir[1] opens into the oesophagus by means of a simple pore in the middle line, the two small latero-dorsally situated reservoirs open laterally into the oesophagus. Beginning at its junction with the oesophagus, the large reservoir (Plate VI, Fig. 1) is seen to extend backward under the alimentary canal as far as the 6th or 7th abdominal segment when completely filled. The anterior portion is narrow, posteriorly it widens into a fusiform sac. The dorsal reservoirs extend dorsally from the oesophagus, outside the large mass of dorso-ventral muscles, between them and the body-wall. The relative size of the reservoirs will be seen by reference to the plate.

Before proceeding to describe these organs more fully it appears necessary to refer to what has been stated with regard to their function by other authors. Grassi (*Studi*, etc. 1901, 2nd edition, p. 108, plate 4, figures 9 and 10) describes and figures them. In his schematic figure the dorsal reservoirs are very small, and the ventral reservoir extends but a little into the abdomen, viz. to near the posterior margin of the second abdominal segment. He refers to the first as lateral "succhiatoi accessorî," to the latter as "succhiatoio principale o stomacho succhiatore o ingluvie." He notes that the sacs have very thin walls, they are lined with a delicate chitinous cuticle, followed by a layer of

[1] According to Packard (p. 305) this sac is always on the left side in Diptera. In our sections it appears median. It appears that such sacs are present in most Diptera and Lepidoptera, where they are falsely called a sucking stomach. In the Lepidoptera they generally contain only air. Newport found the sac filled with food in the flesh-fly and in *Eristalis*, the latter having fed on pollen. Graber also saw food enter the reservoir in flies, the food being coloured. In Hymenoptera the reservoir occurs as a pouch communicating with the oesophagus, and in the honey-bee it is to be distinguished from the "honey-sac," which is the crop or proventriculus. Nevertheless, in bees the reservoir has been seen to be filled with honey.

much flattened epithelium covered externally by muscular fibres. He found them to contain air, mixed with a little colourless fluid, or blood if the insect was examined immediately after sucking blood.

De Grandpré and de Charmoy (1900, p. 21) state that the sacs open into the under side of the oesophageal swelling, that two are situated on the right side (*sic*) and are always less dilated than the third. The oesophageal diverticula, as they style them, contain air-bubbles, at times blood when the stomach is replete therewith. The large diverticulum is on the left side (*sic*), it extends into the abdomen, contains air-bubbles, and it is not infrequently full of blood when the stomach is also full.

Giles (1902, 2nd edition, p. 101) speaking of the oesophagus, says : "It is not uncommon, in dissecting, to bring away attached to it a pair of delicate bags of air-bubbles, the true nature of which will be referred to in the description of the respiratory organs." On pages 103–105, he refers to the "aspiratory vesicles" or, preferably, "the pneumatic sacs," and goes on to say : "This structure is not as has been stated in any way peculiar to gnats, but is, I find, often even better developed in the midges, and other allied insects ; moreover, it is not a median, but a paired structure, and I believe that its size, which has hitherto been absurdly underestimated, is inversely proportional to the size and power of the wings in the different species in which it is found. The reason that it has hitherto been mistaken for a single median sac is that, owing to the pressure of the contained air, the two sacs come to lie one behind the other. They have no true organic connection with the oesophagus and the only reason why they are often brought away attached to it, owing to the fact that the fibrous base of the sacs, which connects them together across the middle line, is divided into two bands, leaving between them an opening through which passes the oesophagus, a little behind the valve in which the latter commences. To the naked eye they look like clusters of minute air-bubbles, and when intact their walls rival in tenuity those of a soap-bubble."... " Instead of being as they have usually been figured, barely larger than the salivary glands, they occupy during life as much or more space than the digestive canal when at its utmost distension. Moreover the more gorged the insect, or the heavier it be with eggs, the larger will these sacs be found, as together they occupy a large space and fill out the entire ventral portion of the body-cavity from the front part of the thorax to the end of the fourth or fifth abdominal segment." Giles does not understand why some should claim the function of these organs to be suctorial, he thinks they act like air-spaces (!) in birds. Continuing (p. 105), he writes, " Into the base of the sacs may be traced large tracheae, and these split up and become continuous with a brush of dichotomously dividing fibres of which the base of each sac is composed." These fibres are chitinous. In a collapsed sac the bubbles of air remain, and under a high power are seen to be entangled in " dilatations of their continuity...in other words these fibres...are extremely elastic and distensible tracheae, which swell out into bubble-containing dilatations wherever their mutual pressure permits of their doing so. Apart from a few loose connective tissue elements, the sacs consist of nothing else but these curiously modified tracheae."

It will be seen from what follows that we do not agree with Grassi in considering these organs suctorial. Their structure points entirely to their being organs which can *expel* fluid, being comparable in this sense to the mammalian urinary bladder. De Grandpré and de Charmoy are in error as to the disposition of the organs in the body, when they place the dorsal reservoirs on one side, the ventral one on the other. Giles claims that there are two sacs, not one, whereas there are three. He denies that they are connected with the alimentary canal, which is very astonishing, for the reason that the simplest feeding experiment or dissection, if skilfully carried out, not to mention serial sections, show absolutely that the sacs are connected with the alimentary canal. It is still more surprising that he attributes to them a function analogous to the air-sacs in birds, and that he has claimed to trace direct connections between the "aspiratory vesicles" as he terms them, and the tracheal system, as it has been impossible for us to find any such connections. In fact the reservoirs compared for instance to the alimentary canal appear to be entirely without tracheae! We can trace no relation whatever between the amount of air in the sacs and the number of eggs it contains.

The name we have given to the three oesophageal diverticula, namely "food reservoirs," indicates what we consider to be their function. There is no evidence that they are suctorial organs, and indeed such a function would be superfluous when we consider the mechanism of the buccal apparatus and the powerful pumping pharynx and highly contractile oesophagus. If an unfed imago is observed under a low power by transmitted light the ventral reservoir can easily be made out, owing to its usually containing numerous small air-bubbles, which move backward and forward owing to irregular contractions of the sac whilst the insect is at rest. In some cases the contractions are very slight, and, as stated, they occur irregularly. Thus in a single insect, observed continuously, 18 slight contractions occurred in two minutes. When fed the coloured fluid food was seen to enter the sac. An hour later, the insect having been left undisturbed, the sac was seen to be contracting powerfully, continuously and more rapidly, 30 contractions being counted in the space of one minute, there being but three pauses. The peristaltic wave travelled backward. In unfed insects 48 hours after their having emerged from the pupal covering the sac was seen to contain much air. On dissection, the stomach was contracted and empty, the three reservoirs frequently being fully distended with air-bubbles.

To facilitate the study of these organs, which for brevity's sake we shall call sacs, imagoes were fed with blood serum and sugar, either alone or together with carmine, or neutral-red. Sometimes the feedings took place alternately on coloured and uncoloured food. Most of these experiments were made upon *Culex pipiens* because we could spare few *Anopheles* for the purpose, both genera however possess the same organs, and they behave similarly.

Experiment 1. Insect fed on sugar-carmine-serum. Killed at once after it had fed very fully, on dissection all three sacs contained food, as did also the intestine. There were 32 very small bubbles in the ventral sac.

Experiment 2. Repetition of the above. No bubbles in the sacs, they contained all the carmine, intestine empty.

Experiment 3. Repetition of the above, insect fed on serum-sugar. Ventral sac half filled with bubbles, 3 bubbles in one small sac, none in the other. Little food in the intestine, nearly all of it in the sacs. This insect had fed but moderately.

Experiment 4. Insect fed on sugar-carmine-serum. Fed again after 2 days with sugar-serum, and killed immediately. No bubbles in the much distended ventral sac, which contained carmine. Small sacs distended with bubbles. Much aggregated carmine in the stomach. No carmine in other portions of alimentary canal.

Experiment 5. Repetition of the preceding: 54 large bubbles, and much carmine in ventral sac. Bubbles distending small sacs. Little carmine in intestine, contained some clear serum, and numerous bubbles along its whole length.

Experiment 6. Insect fed with sugar-carmine-serum. Killed *after one hour*. Some carmine in small intestine, close to stomach. Most of the carmine in the ventral sac, very little in intestine. The carmine in this and other similarly-fed insects could be seen to become deposited at the bottom of the ventral sac, the living insects, resting on the sides of a tube, being viewed under a low power by transmitted light.

Experiments 7 *and* 8. Insects fed on sugar-serum. Killed *after 24 hours*. Moderate amount in the stomach, mostly in ventral sac, bubbles in small sacs.

Experiment 9. Several insects fed with sugar-carmine-serum, killed after 24 hours, showed carmine in intestine down to rectum, besides in ventral sac; at times a few grains of carmine in small sacs.

Experiment 10. Several insects, treated as in 9, were killed *after 48 hours*, there being more carmine in the intestine and rectum.

Experiment 11.   Four insects fed on sugar-serum, killed *after* 48 *hours*.   All contained serum in ventral sac, the small sacs also distended, and containing but a bubble or two, or no bubbles.

Experiment 12.   Insects fed on sugar-carmine-serum.   Killed *after* 48 *hours*.   Large sac contained 24 bubbles, small sacs filled with bubbles.   Carmine in large sac chiefly, also considerable amount in intestine and rectum.

Experiment 13.   Three insects fed with sugar tinged with neutral-red.   Killed *after* 24 *hours*, contents of ventral sac red, many bubbles almost filling it.   Stomach contracted.

Experiment 14.   Six insects fed as in the preceding case were fed again after 24 hours on clear sugar-serum.   The result was very striking.   The contents of the ventral sac were coloured red, that of the stomach yellow, so that there could be no doubt but that the second meal had been almost entirely taken up by the stomach.   This could be easily seen in living insects with the naked eye as they rested on the walls of a glass vessel in which they were contained.   On killing the insects within a few minutes after feeding, and dissecting them, the stomach was found to contain clear serum, the ventral sac coloured serum and bubbles.

The foregoing evidence suffices to utterly disprove Giles's assertion with regard to the function of these organs.   The continued contractions or peristaltic movements of the ventral sac observed in living resting insects indicate that the fluid they contain must for some reason be kept moving.   The bubbles are not as yet explained.   They must have their origin from the outside, that is they must come in through the mouth-parts, either in the process of feeding or afterwards.   During feeding air might very well enter if the pumping action of the pharynx, etc. were continued even for a moment after the removal of the proboscis from the fluid which is being ingested.   It appears reasonable that an insect will seek to clear its mouth-parts and buccal cavity by sucking up the remainder of the food they contain.   It is not impossible moreover that the saliva may be expelled from the tip of the hypopharynx after a meal has been completed, the secretion being drawn back again through the labrum-epipharynx.   The small size usually shown by the bubbles argues in favour of their having been carried in through the small oral aperture.   We propose to pursue this question, which possesses considerable interest.   In the figure shown in Plate VI, Fig. 7 the bubbles are larger than usual.   This may depend upon the nature of the fluid food imbibed, a viscid fluid would

prevent the bubbles combining into large ones in a way which a less viscid fluid would not do.   This also requires study.

When enclosed in the body of the insect the ventral sac is elongated, as shown in Plate VI, Fig. 1, when removed from the body it shortens and widens, for obvious reasons.   When dissected out it continues its peristaltic movement for half-an-hour or more if kept in saline solution. The contractions are due to bands of muscles running transversely but not completely round the sac, and situated at definite intervals along the length of the sac (see Fig. 4).   The contractions are very powerful at the end of the sac, which may contract down so as to have a very small lumen.   The bands situated alongside each other usually contract alternately, the fluid contained in the sac being churned backward and forward.   The system of transverse bands continues along the whole length of the sac until it joins the oesophagus.   The bands terminate by spreading outward into isolated fibrils over the sac.   Delicate diagonally-placed intercommunicating and interlacing fibrils secure a certain amount of shortening in the length of the sac.   The muscle bands are readily shown, by simply allowing a dissected sac to dry outwardly on the coverglass and then staining with an aniline or other stain, the appearance presented being as in the figure.   The small dorsal sacs are also traversed by small bands of muscles, the distribution not being so symmetrical, and the bands but few in number.

If a ventral sac is removed to a slide, and the salt-solution or water in which it suspended is allowed to dry, the sac does not collapse.   The fluid it contains does not evaporate for a considerable time.   Such sacs have been kept for several weeks in the laboratory, exposed to room-temperature and unprotected, and still were found to contain fluid after as long as two months, by which time they had shrunk somewhat.   This is evidently due in part to their being lined with an apparently impervious, but exceedingly delicate layer of chitin throughout. Nevertheless it is not the chitin which prevents evaporation of the contents, for if such a sac is placed in caustic potash so as to remove the soft parts, it will soon dry up when exposed to the air, being completely dried in a few hours.   The chitinous lining is demonstrated most clearly by the use of caustic potash, a sac treated therewith retaining its shape after being macerated for days in that fluid, provided evaporation of the contents is prevented.   No structure could be made out in this delicate chitinous sac.   Similarly a chitinous lining of great delicacy can be demonstrated in the two small dorsal sacs, and parts of the alimentary canal anterior to the oesophageal valve or sphincter.

What we have stated with regard to these sacs appears to justify the conclusion that they are food reservoirs. When feeding, the greater part, or all the material ingested, may find its way into the sacs, and is thence gradually supplied to the digestive canal proper. This is effected by the contractions of the muscle-bands already described, which force the fluid back into the highly muscular oesophagus which doubtless contracts at the pharyngeal end so as to impede the flow towards the pharynx, and contracting backward towards the orifice to the intestine, forces the food into it through the relaxed sphincter.

That blood is also taken up into the sacs has already been stated by Grassi, and de Grandpré and de Charmoy, cited above. This may be of some practical importance. We have seen that insects which were given two good meals collected the first in the sacs, the second directly in the stomach. Of course a certain amount of mixture of the food ingested may take place, and all insects will not behave alike in this respect. Assuming that the first meal is of blood containing malarial parasites, then the parasites might be retained chiefly within the ventral sac, and they would have difficulty in getting out of it. The chitinous lining would prevent the exit of the vermicules, and many parasites would die within the sac; a few might of course be pumped out of the sac later and thus reach the stomach, but by that time many might have died. Of course this is only an hypothesis, but it may possibly explain some of the negative results obtained by various investigators who have failed to successfully infect mosquitoes with parasites. Apart from this, the nature of the food previously ingested by the insect may very well exert a deleterious effect upon malarial parasites entering the food reservoirs subsequently; a matter which appears worthy of consideration by those engaged in infection experiments upon mosquitoes. A suitable opportunity offering, we propose to further investigate the function of these sacs, which does not appear to have been hitherto appreciated. Many insects appear to draw blood directly into the stomach, corpuscles being expelled with a small drop of fluid from the anus toward the end of feeding[1]. Schüffner (1902, p. 93) describes an especially blood-thirsty species of *Anopheles* from Sumatra which ejects 4—5 times as much blood from its anus, whilst feeding, as it requires for a meal, so that if several of these insects are feeding on a hand it is spotted all

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[1] It is of interest to note that Schoo (1902, Feb.?) considers that it is chiefly serum which is ejected. He weighed *A. maculipennis* before (weight 1·9–4·2 mg., average 3 mg.) and after feeding (weight 3·6 to 6·4 mg.), concluding that the amount of blood ingested weighed 1·4 to 2·9 mg.

over with blood.   Schüffner adds that the ejected blood does not coagulate.

Grassi (p. 111) casually states that the air is expelled from the reservoirs just before the insect bores its proboscis into the skin, as a preliminary to feeding.   He gives no evidence as to how this may be accomplished, and it is difficult to judge of whether or no he has made any observations to back the statement.   We have seen in our feeding experiments that bubbles were at times absent immediately after feeding, but this is not always the case.   The mechanism requires further study.   We have not been able to decide how the air is expelled, that is, whether it passes out *per os*, or whether it passes out through the alimentary canal backward.   When the insects rest, as they usually do, with their heads uppermost upon a vertical or inclined surface, or even when hanging by their legs from a surface like a ceiling, all the bubbles in the ventral sac, at any rate, collect towards its exit, and would be readily expelled at will.   The fact that air-bubbles may at times be observed in the mid-gut indicates that some of the air at any rate may be expelled posteriorly[1].   In some insects, evidently in consequence of fermentative processes taking place within the sacs and intestine, these cavities may be greatly inflated.   This condition appeared at times to lead to the death of the insects.

The Oesophageal Valve.

This structure, which appears to be homologous with the proventriculus of many insects, as pointed out by Christophers (1901, p. 14), serves as a valve between the oesophagus and mid-gut[2].   Viewed externally the structure is seen to produce a marked annular thickening of the intestinal wall.   It does not appear to be lined with chitin as are the preceding structures.   The thickening is partly due to powerful annular muscles which act as a sphincter, occluding the lumen by their contraction, and rendering it patulous when they are relaxed for the passage of food from the oesophagus.   The thickening is also due to the intussusception of the gut at this point, the invagination protruding into the tubular mid-gut, somewhat after the manner of the cervix uteri into the vagina in man.   Attached to this portion of the alimentary

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[1] According to Packard (p. 324) Dragon-flies, Orthoptera and Lepidoptera swallow some air with their food.

[2] Weissmann (1864, cited by Packard, p. 311) already regarded the proventriculus of flies as an intussusception of the oesophagus.

canal are six small protuberances, which are more or less marked in
different insects, these are reduced caecal appendages which are well-
marked in the adult larva, where they are also distributed in a circle
about the gut. They are indistinct in the imago, whereas in the larva
they form elongated cavities communicating directly on one side and
by a pore with the lumen of the gut. Grassi says there are many, and
figures the caeca to the number of nine, whilst Christophers (p. 13) says
they are absent. The lumen of the valve opens directly into that of the
mid-gut. The sphincter, just referred to, may continue to undergo
rhythmical contractions for two or more hours after the gut has been
removed to salt solution, the parts not being subjected to pressure from
a coverglass.

The Mid-gut (or "Chylific Ventricle"), including the "Stomach."

(Plate VI, Figs. 1 and 7, Plate VIII.)

The mid-gut runs as a simple straight tube from the oesophageal
valve at about the level with the first pair of legs to about the level of
the posterior limit of the sixth abdominal segment, as also found by
Grassi. The relative position with regard to the parts of the exo-
skeleton just mentioned will vary somewhat according to the degree
of distension of the gut with food. The mid-gut can be compared to
a long-necked flask, the anterior portion of which is narrow and tubular,
the posterior portion dilated. It is the posterior portion of the mid-gut
which is usually styled the "stomach" by medical writers, and the term
is convenient because of brevity, for it is in this portion of the mid-gut,
chiefly in its posterior two-thirds, that malarial parasites develope in their
insect host. We shall therefore refer to this portion of the mid-gut in
future as the stomach. The anterior tubular portion of the mid-gut
("colo del stomaco" of Grassi) has a slight dorsal flexure, to give room
to the ventral reservoir described above. The dilatation or stomach
begins on about a level with the second abdominal segment. When
there is food in the mid-gut it accumulates in the stomach. Grassi
observed that after a meal of blood the corpuscles accumulated in the
posterior $\frac{3}{4}$ of the stomach, the serum being contained in the anterior
portion. Giles (1902, p. 102) states that in the recently emerged imago
the stomach may contain remains of food ingested by the larva.

The mid-gut has a similar structure throughout. It is not lined by
chitin, as can be seen when the gut is macerated in caustic potash.
There is no trace of the chitinous tube, known as the peritrophic

membrane, which is found in the larva. The change of food from solids to fluids no doubt accounts for this.

According to Grassi the internal coat consists of a delicate *cuticula,* which he figures and describes (Plate VIII, Fig. 2). The cuticula is figured but not described by Christophers. Packard states that it is always present in insects. The second coat, which forms the greater part of the thickness of the wall, is made up of a single layer of large cylindrical or cubical *epithelial cells,* with large oval nuclei. The form of the epithelium may be altered by pressure exerted by the food within or parasites without, as has been shown by Christophers and Grassi. When the stomach is distended the cells are flattened. Pressure from parasites causes a distortion of the cells corresponding to the form of the parasites which press upon them. Christophers writes (p. 13) of the epithelial cells :—" They have a finely-reticulated protoplasm, which stains more deeply toward the free border. Stained with Heidenhein's haematoxylin alcohol-hardened specimens are seen to contain numerous stained granules collected especially in the outer portion of the cell. They are especially abundant in the anterior portion of the mid-gut. They have also very frequently a number of small clear vacuoles (droplets) which become more frequent and of larger size towards the free border of the cell. The most marked feature of the cell is the clear striated border which is present in all the cells of the mid-gut, but absent in all other portions of the alimentary canal. The striated border is best marked in the undistended organ, and becomes almost invisible in the fully distended state when the cells are much flattened. The nucleus of these cells is large and centrally situated. The chromatin is arranged in small stellate masses arranged circumferentially and centrally and connected with one another by fine threads of chromatin. There is a body which stains less deeply generally to be made out (karyosome) in the centre of the nucleus. Occasionally young cells are seen near the basement membrane." Our observations accord with these.

External to the epithelium lies an elastic basement membrane, which Grassi styles the *elastic-muscular tunic,* for the reason that the muscular fibres appear to lie embedded within its substance. That the basement membrane is elastic is indicated by the fact that it stretches when the intestine is distended and the epithelium upon it is flattened. It appears to be structureless. The membrane can be removed, as Grassi has shown, from the epithelial layer, thus facilitating the examination of the coat for malarial parasites, the view being impeded by the epithelial cells with their large, darkly-staining nuclei. The

bands of muscles cannot be separated from the membrane, for the reason that they are apparently ensheathed in its substance. Grassi (1901, pp. 175—176) is of the opinion now that the amorphous substance forming this outer tunic constitutes what has hitherto been considered the capsule of the oocysts of the malarial parasites, and this for the reason that the "capsule" stains like the basement-membrane and is continuous therewith. This appears reasonable because of the probability that the parasites nourish themselves upon the products of digestion which pass through this membrane from the mid-gut to the coelom.

Muscular fibres run around and longitudinally upon or within the amorphous layer, forming a loose network (see Plate VIII, Fig. 1) such as was already figured in Ross's earlier papers. Where the intestine is contracted (see Plate VI, Figs. 1 and 7) the circular fibres throw the surface into a large number of transverse folds, each muscle-band forming an annulation. When dilated with food the play of the separate muscle-bands may be observed in the excised stomach in salt solution. When the bands contract the stomach is indented by the separate bands. Ovoid when dilated, the stomach may contract about the centre, assuming the form of an hour-glass, etc. The muscular bands are very long and of remarkably uniform width. Christophers (p. 13) states that all the muscular bands of the alimentary canal are striated[1]. The outer surface of the mid-gut shows numerous large branched cells in which the small tracheae end, and from which bundles of exceedingly minute structureless air-capillaries pass into the wall of the mid-gut. These cells are often well shown according to Christophers, in specimens stained with gold chloride. Such cells occur throughout the viscera in connection with the tracheal endings and have been described in other insects (see figures in Packard, p. 436). If we except the anal glands, the mid-gut is more plentifully supplied with tracheae than any other portion of the alimentary canal, large dividing spiracles occurring plentifully, especially over the stomach, the smallest spiracles measuring anywhere from 2 to 6 μ across. The fat-body which is more or less marked according to the stage of nutrition of the insect, is not organically connected with the

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[1] This requires further study. Packard (p. 316) states of some insects that the inner (circular) layer of muscles is unstriated, the outer (longitudinal) striated. On p. 324 occurs the remarkable statement : "Suctorial insects draw in their liquid food by the contractions followed by the dilatations of the mid-intestine," a conception which is obviously false.

mid-gut about which it may in part lie.   The gut seems to lie freely in the body-cavity, being obviously in a measure kept in place by the tracheae which arise from its surface and pass outward to the larger air passages and stigmata.   It is not impossible that delicate muscles, such as are described by Lyonet (Packard, p. 297) as " retractores ventriculi," may also afford additional loose fixation, but we have been unable hitherto to detect them.

The Hind-Gut.

The hind-gut begins at the junction of the Malpighian tubes with the end of the mid-gut.   The lumen, which is wide in the stomachic dilatation of the mid-gut, here suddenly narrows.   The hind-gut is divided into the ileum, colon, and rectum, and ends with the anus. The ileum curves dorsally, the succeeding bend in the intestine representing the colon.   The rectum is dilated into a sac, but narrows where it approaches the anus.

The *ileum* is very short.   It may be somewhat dilated near the mid-gut.   It is lined with flattened epithelium.   It is very transparent, the contents being readily seen through its walls.   As Grassi (p. 109) states, it is lined with a chitinous cuticula, which appears thickened in undulating lines or ridges.   When dilated he notes that the ridges, which are very close to each other, are due to cuticular thickenings on a level with the lines which mark the anterior and posterior margins of the epithelial cells.   The ridges to a certain extent invade the contiguous cells.   We have seen this portion of the intestine undergo active contractions for hours after removal from the body, its activity in this respect being comparable to what has been noted in the oesophageal valve.

The *colon* succeeds the ileum, without there being any line of demarcation.   The colon is lined by a single layer of epithelial cells of cubical form.   Christophers (p. 14) notes that the nuclei of these epithelial cells are similar to those of the mid-gut, although they possess a more open arrangement of the chromatin.   " The protoplasm is finely reticular, and stains less deeply than the cells of the mid-gut. Stained with Heidenhein's haematoxylin no granules are present as in the cells of the mid-gut.   They have no striated border."   The muscular coat is well developed in this region, showing a well-marked fenestration, or crossing of fibres.

The *rectum* (Plate VI, Fig. 2) forms a spacious oval chamber into

which the colon suddenly opens. Its lumen is diminished by the protrusion into it of six large ovoid papillae[1]. The cavity is lined with flattened epithelium. Each of the papillae consists of a number of large cells, modified from the ordinary lining cells of the rectum. A bundle of minute tracheae passes up through the centre of the papilla, between the cells (see Plate I, Figs. 2 and 3), the tracheae being distributed from the apex backward, and apparently uniting again into a bundle, returning to the same large trachea whence they had their origin. The papillae are covered with chitin.

The rich supply of tracheae to the rectal papillae indicates that these organs must fulfil some active and important function. What this function is is not clear. Such papillae are widely distributed amongst insects. Miall and Hammond write (1900, p. 107), "Chironomus has two, most other Diptera four, Pulex, most Hymenoptera, Neuroptera, and Orthoptera six, Lepidoptera 60—200, Coleoptera and Hemiptera none. They are absent in larvae with few exceptions." The abundant supply of tracheae and the analogy of the anal respiration of such insects as the larvae of dragon-flies have been put forward as supporting the view that the rectal papillae are respiratory organs. Others believe they function as glands, but as Minot points out their structure lends little support to this view. Fernald (cited by Packard) regards them as valves, but although the papillae must offer some obstruction by their presence to the passage of the intestinal contents, they have nothing of the usual structure of valves, at any rate in *Anopheles*.

The rectum narrows just before the anus, the narrowed tube being well beset with muscles, both longitudinal and circular. The anus is just ventral to the orifice of the reproductive organs, and is guarded by two short lateral papillae. It is "situated in the last segment of the body, under the last tergite or suranal plate," a position which Packard (p. 297) says is invariable in insects.

The Malpighian Tubes.

The five Malpighian tubes, which are already fully developed in the larva, open into the hind-gut at the same level, at its junction with the termination of the mid-gut. The fact of their containing chitin points

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[1] Giles (2nd ed. 1902, p. 103) is evidently in error when he states that there are four anal papillae. He states that they are connected by short ducts to the intestine, and that they probably secrete some "fluid accessory to digestion."

to their being derived from the embryonal proctodaeum. They lie bathed in the fluids of the haemocoel and are slightly coiled, making one or two loops, so that in cross section the same tube is not infrequently cut twice. As a rule the number of Malpighian tubes is even in insects; Packard (p. 350) citing *Culex* and *Psychodes* as "remarkable exceptions" in possessing five. *Anopheles* we see, also possesses five, and if Eysell (Oct. 1902, p. 341) is right *Aëdes* possesses a similar number, for he distinctly states of *Aëdes cinereus* Hoffmg., that the intestinal canal and its appendices are exactly as in *Anopheles* and *Culex*. The usual number of tubes in Diptera is four.

Commencing at the entrance of the tubes in the intestine, they are seen to be lined for a short distance by cells continuous and similar to those of the gut. Secretory cells however soon appear, which presumably excrete the waste nitrogenous matter from the body. Each of these cells is very large, the nucleus being conspicuous. The tubules consist of a double row of cells, arranged alternately and enveloping the excretory duct. The alternate arrangement of the cells gives the tube a wavy appearance. The lumen of the tube is usually somewhat flattened, being lined by chitin, which is supposed to be perforated by porosities. In some sections the lumen is dilated, apparently owing to excreted matter. The cells rest upon an apparently structureless basement-membrane.

No muscular fibres appear to be present in these organs. They usually have a pale yellow colour when viewed by transmitted or reflected light, the colour being due to the matter excreted. Packard (p. 352) states that the colour varies according to the nature of the food, turning red for instance in certain insects when fuchsin is mixed with their food. Uric acid and other renal products have been found in these organs, as also concretions, etc. The Malpighian tubes are richly supplied with tracheae.

The Salivary Organs.

The salivary apparatus in adult mosquitoes is not connected with the alimentary canal[1]. The saliva issues from tip of the hypopharynx (*Journal of Hygiene*, Vol. I, p. 464, and Plate IX) when the insect feeds. Examined under a low power the hypopharynx appears to possess a duct, situated centrally, and running from near its tip to its

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[1] Giles (2nd ed. 1902, p. 100), speaks of the salivary duct as "a chitinous tube prolonged from the lining of the buccal cavity," this being incorrect.

base, a duct being figured in the plate referred to above. When cross sections of the hypopharynx are examined under a high power (magnification 500 or over), the supposed duct is seen to be a groove, or "salivary gutter" as it has been styled by Annett and Dutton. We have figured a cross section of the hypopharynx highly magnified on Plate VII, (Fig. 6), the section having been made about midway along its length. It will be seen that the groove in the chitin serves the purposes of a duct, for, although open dorsally, delicate chitinous lamellae arch over the opening, and overlap, practically closing it. The groove is of remarkably uniform width throughout the length of the hypopharynx; the lumen, in several specimens, measured 5·8 to 6·6 μ.

At the base of the hypopharynx we find a very interesting structure, connecting the common salivary duct and the groove (Plate VII, Fig. 6a). It has been described as the "salivary receptacle" by various authors, including Macloskie (1888), and Annett and Dutton (1902); the latter appear to have been the first to rightly understand the mechanism. The structure is more than a receptacle, it constitutes a *pump*, the mechanism of which corresponds to that of the pharyngeal pump in a sense, that is, it depends upon the action of powerful voluntary muscles which overcome the elasticity of a chitinous membrane which, when released by the muscles becoming relaxed, rebounds or returns to its original form, as a bow does when the pull on the bow-string is released. The chitinous mechanism is best studied in dissected parts which have been treated by caustic potash. It is very difficult to cut the valve in sections for the reason of its being highly chitinized, the structure being frequently torn out of its situation by the knife. It will be seen then that the common salivary duct ends (lumen 5 μ) in the centre of a chitinous membrane, the junction being strengthened by a chitinous thickening of annular form. The membrane is continuous with a highly chitinized cup, which tapers anteriorly, and is continuous with the hypopharynx, an opening therein connecting it with the groove described above. We shall not here consider the other chitinous structures surrounding the salivary pump. As is shown in Fig. 6a spicules of chitin occur about the duct on the pump-membrane, these serving for the attachment of the powerful muscles presently to be described. The thickened chitin surrounding the membrane is flattened on its dorsal surface which is applied to the floor of the buccal cavity. The pump-membrane is covered in the centre by the insertion of two stout bundles of muscle-fibres which pass backwards, parallel

with one another, to their origin on the anterior surface of the chitinous flange which projects ventrally from the floor of the buccal cavity. (Plate VII, Fig. 5.) When the muscles contract a partial vacuum is produced within the cup, saliva flows in from the glands, and when they relax the membrane rebounds forward, driving the saliva out of the cup into the salivary channel along the hypopharynx. In an earlier part of this paper we referred to these muscles as retractors of the hypopharynx (Vol. I, p. 464), a statement which must now be withdrawn. The relation of the base of the hypopharynx to the bases of the other mouth-parts admits of no free movement of this organ. The hypopharynx is probably withdrawn with the other piercing stylets, this withdrawal being mainly effected by the bracing action of the insect's legs lifting the head and body away from the object bitten as we already stated elsewhere (Vol. I, p. 467). We believe this to be the case from observations on living insects which have been allowed to suck our blood, being closely watched during the process. Proceeding backward from the salivary pump the common duct (lumen $4\,\mu$) passes beneath the buccal cavity to a point beneath the valve which separates the buccal cavity from the pharyngeal pump. Here it divides into two ducts of similar structure (lumen $3\,\mu$) which run closely side by side, beneath the infra-oesophageal ganglion and ventral nerve-cord along the ventral wall of the neck into the thoracic cavity, where they diverge and branch into the salivary glands.

The glands appear in cross sections close to the neck, and reach back to a little beyond the oesophageal valve, being situated laterally with regard to the oesophagus. Each branch from a secondary duct divides into three smaller ducts (lumen about $2\cdot5\,\mu$) immediately before entering a corresponding number of glands, see Plate VI, Fig. 6. Taking the one set of glands, we see, in cross sections, that they are arranged in a triangle at first, one gland being dorsal, the other two ventral and close to each other (see Plate VI, Fig. 8, where the dorsal or *central* [see below] gland is on the left). The glands then gradually shift their positions, so that the dorsal gland comes to lie between the two previously ventral glands, these being placed, the one ventrally, the other dorsally. This is due to the glands having to accommodate themselves to the position of the powerful thoracic muscles which move the wings. The glands are surrounded to a varying degree by the fat-body.

When the salivary glands are removed from the mosquito (methods to be described later), they present an appearance such as is figured in Plate VI, Figs. 5, 6, and 7. They are very large in proportion to the

size of the insect. If the dissection is skilfully performed the two sets of glands with their (secondary) individual ducts and common duct are brought away together. At times a certain asymmetry is observed in the glands, as figured (Fig. 7). In Fig. 5 it will be seen that the central gland is smaller than the lateral, and this is also shown in Fig. 7, where this gland (as can be seen by looking closely at the junction of the three ducts quite near the glands) is displaced to the left. This smaller, central gland ("tubulo intermedio" of Grassi), as we shall for convenience call it, is the one which appears to occupy a dorsal position in sections near the neck, an intermediate position further back, with regard to the two other glands. The central gland differs also from the others histologically, as we shall presently see.

The size of the glands as a whole varies with the dimensions of the individual insect. The glands are considerably larger in the female than in the male, the salivary pump being moreover small in the male. Measurements made of the fresh salivary glands of a female insect of average size showed a width of $85\,\mu$, the lateral glands being $880\,\mu$, the central gland $510\,\mu$ long. In a male, the largest gland measured 51 by $212\,\mu$. The measurements were made longitudinally through the axis of the gland. With the exception of Grassi (p. 110), who states that the lateral salivary glands of the female *A. maculipennis* measure 1 mm. in length, we are not aware that the size of these glands has as yet been determined. It will be seen that Grassi's measurement agrees fairly well with ours of the lateral gland, allowing for variations in the size of the insect and contraction of the gland, which may be observed to take place after its removal to salt solution, especially when the anterior portion is dilated as shown in Fig. 5 (Plate VI), the drawing having been made immediately after removal.

The common duct and secondary duct are chitinous and surrounded by a well-marked sheath[1], within which they pursue a slightly serpentine course which indicates that the substance of the sheath is not firm. Outwardly the sheath possesses a basement membrane, and within, cells with clearly staining nuclei. In unstained specimens the substance around the duct itself appears slightly granular and homogeneous. These ducts throughout their length, and for a little distance along the three branches of the secondary ducts into each set of glands, show a structure somewhat similar to what is seen in the tracheae. Some

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[1] See Plate VI. This is not shown in the diagrammatic cross-sections of the head in Plate VII, Figs. 1–3, nor in Fig. 4.

authors have described the chitinous ducts as showing spiral thickenings, as in the tracheae, but this is not the case.   On examination with a high power the thickenings are seen to be incomplete hoop-like bands. These thickenings project slightly outward like the tracheal cartilages of mammals.   Like the tracheae the ducts possess a considerable degree of elasticity, as can be seen in the process of dissection.

The presence of the chitinous ducts leading to and into the glands is explained by the fact that the salivary glands are developed like the fore-gut from the embryonal proctodaeum.

The salivary glands of a mosquito (*Culex taeniorhynchus*) were first described by Macloskie (1887) who figured them, dissected out, and showed the arrangement of the ducts in longitudinal section through the head.   He noted a difference in the appearance of the central gland, to which he attributed the toxic action of the salivary secretion of the mosquito, there being no obvious ground for such an assumption.   His figure shows that a chitinous duct runs through the axis of the gland to near its base.

Considerable attention was drawn to these glands by the investigations of Ross, who first demonstrated the presence of the sporozoites or " blasts " of the avian malarial parasite (*Proteosoma*) within the cells of these glands.   Subsequently Grassi, Bignami and Bastianelli found the sporozoites of human malarial parasites in a similar situation in **Anopheles** infected with malarial blood.   The parasites of aestivo-autumnal malaria, as also of tertian and quartan ague, have been found as sporozoites within the cells of the salivary glands, by the Italian observers, as also by Ross and others since.   See Fig. 3, Plate IX.

In their essential structure the three glands are similar, consisting of acini.   Each acinus is surrounded by a basement membrane, which can be clearly demonstrated in fresh glands by placing them in non-isotonic fluid so that the epithelium which rests upon it contracts away from the enveloping membrane.   It may also be seen at times in hardened sections.   The membrane is extremely delicate and apparently structureless, although de Grandpré and de Charmoy (p. 29) claim to have found muscle-fibres, both longitudinal and circular, therein.   A single layer of epithelial cells rests upon the basement membrane and surrounds a central lumen, which can be made out in fresh glands, through the transparent structures.   The lumen is occupied by a chitinous intra-glandular, or intra-acinar duct, communicating anteriorly with the secondary duct, which is joined at once by the three

intra-glandular ducts at one point. As was first pointed out by de Grandpré and de Charmoy (1900, p. 21) there is a difference with regard to the intra-glandular duct in *Anopheles* and *Culex*. These authors, as also Christophers since (1901), have figured the difference which they describe. As Macloskie showed, the duct in the central gland of *Culex* possesses a uniform width down to the base of the acinus. In *Anopheles* it broadens soon after entering the acinus. In other words the gland is sacculated in *Anopheles* and not in *Culex*. Whereas Eysell (p. 341) states that there are also three glands in *Aëdes cinereus* Hoffmg., he says nothing regarding the structure of the ducts in this insect. In fresh glands mounted in glycerine, which increases the transparency of the structures about the duct (Plate VI, Fig. 6), the intra-glandular ducts are seen to at times give off short lateral branches, which may be longer if the gland possesses a small lateral acinus, such as we have figured. These branches appear to terminate blindly, as do the main intra-glandular ducts in *Culex*. On the other hand the chitinous lining of the duct would appear to end in *Anopheles* before it has reached down half-way into the lateral glands, and even sooner in the central gland, as is shown in our figure. Either the chitin actually ends at the points figured, or it abruptly grows so thin that it can no longer be discerned in the posterior portions of the acini. We are not aware that this has as yet been noticed. Grassi (p. 111) states that he was once able to distinguish minute pores in the delicate cuticula which forms the intra-glandular duct, on examination of fresh specimens. Giles (p. 101) examining these ducts in an undetermined species of *Culex*, writes "it can be distinctly made out that, minute though it be, this chitinous wall of the tube is pierced by spirally arranged minute perforations, each corresponding to the point of origin of a cell." His figures of the gland and ducts do not agree in several respects with what we have observed in glands of *Culex pipiens* and *C. annulatus*, and we have been unable to determine any "spiral arrangement" of the pores in question. We have detected the minute pores both in *Anopheles* and *Culex*, especially in ducts treated with caustic potash, the soft parts of the glands which had previously been dissected out being thus removed. To see these minute pores requires careful illumination and a magnification of about 1000. Viewed under such conditions the intra-glandular duct is seen to be perforated by minute circular pores, and to be roughened on the outside by extremely delicate chitinous spicules, which possibly serve for the attachment of epithelial cells, it being apparently true as Giles

states that individual cells may remain adherent to the duct in glands which have been injured.

The individual epithelial cells can be made out in unstained glands, to which they give a beaded appearance. The lateral glands may broaden toward the front, this being apparently due to the glands being filled with secretion, the gland serving as a reservoir, in the absence of a true receptacle. It is a misnomer to refer to the pump already described as a salivary receptacle. The central gland narrows anteriorly to a neck (Fig. 5, Plate VI).

The *lateral glands* possess a similar structure histologically. The acinus is more or less filled with secretion (Plate VI, Fig. 8), and the cells surrounding the lumen broaden toward the base, near which the nucleus lies. These glands, when examined stained or unstained, are seen to differ from the central gland. Their secretion certainly does not stain with eosin as does that of the central gland, as has been shown by Grassi, a fact we can confirm. The bulk of the acinus and consequently of the epithelial cells is composed of the mass of secretion, the cell protoplasm and nucleus being forced backward by it against the basement membrane. When a fresh gland is crushed the secretion escapes in the form of clear refractile drops. In hardened sections the clear secretion, as has been pointed out by Christophers (p. 15), appears as a granular mass filling the greater portion of the cell. It stains poorly with haematein, and exhibits a coarse reticulum and isolated globules, probably, as he says, due to precipitation or coagulation of the secretion by alcohol. Grassi (p. 111) found the portion of the glands near the exit of the duct to contain a secretion which was more refractive than that contained towards the blind end of the acinus. Stained with haemalum the portion toward the exit of the duct became diffusely coloured, this not being the case in the posterior portion. In glands preserved directly in absolute alcohol as also in fresh preparations when examined late, the secretion appeared granular or showed delicate filaments, which must be considered artefacts. As both Grassi and Christophers point out considerable variations exist in the appearance of the granular secretion in different mosquitoes and in one and the same gland. We also find as stated by Christophers that the greater portion of the gland is filled with granular substance, and for this reason this author has referred to these glands as belonging to the " granular type " in contradistinction to the following, which he styles " clear or colloid type." In cross-sections through the thorax, stained in various ways, these differences between the central and lateral glands are

brought out strikingly, it being possible to compare the glands which lie side by side. In Plate IX, Fig. 3, a small part of a lateral gland is shown above the central gland which contains parasites.

The *central gland*, as we have seen, is shorter than the lateral ones, and narrows anteriorly where its duct emerges. Christophers notes of the fresh gland that the cell contours are not so distinct nor the secretion so refractive (Grassi says it is more refractive) as in the lateral glands. The secretion almost entirely fills the cells, which however contain more protoplasm than do those of the lateral glands. The hyaline secretion stains intensely with eosine, Heidenhein's haematoxylin, and also with haematein. The cells at the neck, as Grassi pointed out, do not stain with eosin, this being striking as the secretion in this portion stains therewith. Christophers notes that the nuclei of the secretive cells are less inclined to present degenerative appearances than in the lateral glands. He found that the secretion in the dilated central duct at times presents an appearance of faintly stained sporozoites, this being apparently something like the artefacts Grassi noted, as stated above. Christophers (p. 16) states that in freshly hatched insects the cells in both types of acini contain a large centrally situated nucleus, the protoplasm containing a large number of coarse granules staining with haematein. " This is the commencement of the large mass of secretion which, in the mature gland, occupies the entire cell."

Both Grassi and Christophers have studied the glands of insects which have fed and have remained unfed, but noted no especial difference in the appearances. It would therefore seem as if but a very small amount of secretion were given off. Viewed in fresh specimens Grassi thought to note that the central gland seemed somewhat more contracted (narrower) in insects which had fed than in unfed insects.

We have not as yet had an opportunity of studying an appearance of the glandular epithelium, casually referred to by de Grandpré and de Charmoy (p. 26), namely, "les prolongements ciliformes de l'épithélium que Bordas décrivait dès 1894 chez plusieurs familles d'Hyménoptères et en 1896 chez les Orthoptères et que Lecaillon vient de retrouver en 1899 chez le *Culex pipiens*." These "prolongements ciliformes," it appears, are not vibratile and are seen in thin sections stained with magenta-red and afterwards with indigo-carmine. Each process penetrates into the cell and connects with a deeply-staining body. No reference is given to the Journal where Lecaillon's paper appeared.

The Effects of the Salivary Secretion.

The effects of mosquito-bites are well known to most people. The method of feeding has been described elsewhere (this *Journal*, Vol. I, p. 467). Immediately after the insect has withdrawn its stylets, a small area about the puncture whitens, and after a minute or two becomes pink, swelling commencing. Soon afterwards the spot begins to burn and itch, the itching being relieved by pressure, only temporarily relieved and subsequently increased by scratching. In tropical countries especially the scratching of the spot may lead to the formation of wounds which heal badly, possibly owing to infection through the lesion. It is notorious that new-comers are more affected than aborigines in mosquito-infested countries. There is evidence that a certain degree of immunity may be acquired to the poisonous action of the mosquito saliva. Some persons show a high degree of susceptibility, others apparently a considerable degree of natural resistance to the effects of the toxin. Several cases of death due to being very severely bitten are recorded. Where the bites are numerous the swellings due to each bite become confluent, and there may be considerable oedema. For instance in a hunting expedition in Canada one of the writers was so severely bitten some years ago that one eye was closed, as also one ear through oedematous swelling, the face on one side being so swollen as to render him utterly unrecognizable. Experiments conducted here in England by one of us with *Anopheles maculipennis* have demonstrated what had been proved elsewhere for this insect, that its saliva is distinctly toxic. An hour or so after being bitten, provided the parts are not rubbed, the itching may cease, although the elevation around the puncture does not subside. At night-time, and in the morning, doubtless owing to the heat of the bed, the spots begin again to itch and grow red. Bites subjected to the friction and heat of clothing also tend to remain irritated. In some cases this irritability of the spots was renewed every night for a week or ten days, and all trace of the bites had only disappeared after 11 to 14 days. Of course the intensity of the effects and their duration vary with different individuals. It should be noted that in these experiments the insects were allowed to feed and afterwards withdraw their stylets without being disturbed, this being done for the reason that it is not infrequently asserted, as we see without reason, that if the insect is allowed to feed without being disturbed there may be no after-effects

whatever, the mosquito supposedly having time to withdraw the saliva it pumps into the wound.

Leeuwenhoek thought that the inflammation following the bite of *Culex* was due to the nature of the wound inflicted. Réaumur (1738), who cites him, considered that the effects were due to a toxic fluid injected by the mosquito with the object of increasing the blood-flow. Macloskie (1887) considers the salivary secretion " seems by inflaming the tissues to determine a flow of blood and also to prevent the coagulation of blood or other proteid " on which the insects usually feed. The blood, he notes, subsequently coagulated in the insect's stomach. It has also been suggested by others that the secretion might prevent the coagulation of the blood, which would clog the mouth-parts, to the detriment of the insect.

The problem was not approached experimentally, until last year when one of us (G. H. F. N.), seconded by Dr Graham-Smith, made some preliminary investigations which will be continued this year. Fragmentary though they be, the data recorded do not support the hypothesis that the salivary glands, at any rate of *Culex pipiens,* contain a substance which prevents coagulation.

Some Experiments with Emulsions of the Salivary Glands of Culex pipiens.

1. Six sets (36 acini) of glands were dissected out of freshly killed insects, and placed in a drop of salt solution. The drop was allowed to dry, it being thought that the salt crystals would facilitate the grinding up of the glands with the end of a small glass rod, this being done under microscopic control. After grinding up, a small drop of water was added of the size of the original drop of saline, and an equal volume of human blood taken from the clean finger-tip was quickly mixed therewith, and the whole drawn up into a capillary tube. Clotting was not prevented and no haemolysis occurred. Salivary gland emulsion added to a dilute suspension of corpuscles did not lead to haemolysis.

2. Twenty-two sets (172 acini) of glands were emulsified as before, and added to guinea-pig blood, both pure and diluted. The result was the same as before.

3. Twenty-one sets (160 acini) were ground up with powdered glass-wool on a hollow-ground slide with a couple of drops of salt solution. The glass was removed by centrifugalization. Human blood, both pure

and diluted, was added to the gland-emulsion, and drawn up into capillaries. The blood clotted and was not haemolysed.

4. After 24 hours the contents of these tubes were injected subcutaneously in two places into a guinea-pig, but no swelling occurred at the seats of inoculation.

5. A capillary containing some of the above gland-emulsion alone was placed beneath the skin of a rabbit's ear and broken. One-half of the tube removed after 24 hours was found to contain unclotted blood, haemolysis had occurred, some corpuscles appearing as shadows, others being crenated. There were many leucocytes at the entrance to the tube. These effects may have been due to *Streptococci*, which were found microscopically in the tube-contents.

As stated above, these experiments are of a preliminary character. As soon as the season is sufficiently advanced they will be continued. It remains to be proved if the salivary secretion ferments starch or not ; it appears to do so in some insects.

Concluding Note.

When the first part of this study of the " Structure and Biology of *Anopheles*" appeared in the first volume of the *Journal of Hygiene* the writers did not expect that it would take so long to reach a conclusion. The delay in publishing the different parts has been partly due to lack of material, especially during 1902, when *Anopheles* proved exceptionally scarce. The descriptions we have published apply to matters regarding the anatomical features and biology, which should interest medical readers. We have not as yet described the nervous, circulatory, respiratory, and reproductive systems, nor the structure of the eye, etc. A description of these appears somewhat too technical for the purposes of this *Journal*, and for this reason it will be given elsewhere. In the meantime we would add that we shall probably from time to time publish additional observations on the subject of *Anopheles*, confining ourselves however to matters of medical interest. Such communications will take the form of separate papers. This paper may therefore be regarded as concluding the " Studies in relation to Malaria " which have appeared serially in this *Journal*.

The "Studies in Relation to Malaria" comprise Part I: "The Geographical Distribution of Anopheles in relation to the former distribution of ague in England" (*Journ. of Hygiene*, Vol. I), and Part II: "The Structure and Biology of Anopheles" (*Journ. of*

Hygiene, Vol. i—iii). The investigation was originally planned to include a Part III : relating to malaria-infection experiments upon *Anopheles* indigenous to England. It is to be hoped that these will be ultimately carried out successfully. Several attempts have been made in the last two years to infect *A. maculipennis* with malarial parasites, but they have failed. These experiments were made by one of us (G. H. F. N.) in conjunction with Mr Strangeways of Cambridge, and members of the staff of the London School of Tropical Medicine, the late Mr Patrick Thurburn Manson, and Dr C. W. Daniels.

The announcement that the *Index Medicus* will be revived this year relieves us of the necessity of giving further bibliographies of recent literature, such as follow in the Appendix to this paper. It has appeared desirable to publish these bibliographies in view of the scattered nature of the literature. The labour spent in their compilation should facilitate the work of others who are engaged in the study of malaria and its prevention, as also in mosquitoes.

We gratefully acknowledge the able assistance of Mr Edwin Wilson, F.E.S., in the work of illustration. His technical skill and keen interest in the subject have materially furthered the morphological studies on *Anopheles*.

The expenditures entailed in the prosecution of the "Studies in Relation to Malaria" have been largely defrayed by grants from both the Government Grants Committee of the Royal Society, and from the John Lucas Walker Fund, Cambridge.

EXPLANATION OF PLATES.

Illustrating the paper of G. H. F. Nuttall and A. E. Shipley on
"The Structure and Biology of *Anopheles maculipennis*."

PLATE VI.

Fig. 1. Schematic longitudinal section of a female insect showing the relations of the various parts of the alimentary tract to each other, and to the exoskeleton, as also the salivary glands of one side with their duct joining the common duct, which is prolonged into the hypopharynx. Ventral reservoir filled, the stomach contracted. One of the dorsal reservoirs is cut off near to where it joins the oesophagus.

Fig. 2. Longitudinal section through the posterior portion of the abdomen, showing a portion of the colon, the rectum with the papillae of one side, as also voluntary muscles about the anal orifice.

Fig. 3. Anal papilla as seen in fresh specimen, showing distribution of tracheae therein.

Fig. 4. The ventral reservoir, filled with food, has been removed to a slide, and allowed to dry externally, being then stained with fuchsin. The sac had broadened through lack of its usual support, but nevertheless measures roundly 4 mm. in length, the neck being included. The muscle-bands do not completely encircle the sac.

Fig. 5.   Freshly dissected glands (from one side) of a female insect.   Viewed in salt solution without a coverglass.   The central gland is small, and narrows to a neck where it joins the secondary duct.   The lateral glands broaden anteriorly.   After about 15 minutes these glands grew narrower anteriorly (secretion expelled ?).   Note the undulating course of the chitinous duct within its sheath, both in this and the succeeding figure.   The junction of the secondary with the common duct is also shown in both figures.

Fig. 6.   The same as the preceding, the specimen having been mounted in glycerine, which has rendered the soft parts hyaline and permits a study of the duct-structure. The central gland has been displaced to the left of the beholder, as can be seen by the torsion of its duct where it joins the secondary duct together with the intra-glandular ducts of the lateral glands.   The chitinous duct within the central gland is shorter than in the lateral glands, but in all three there is a terminal broadening, and the ducts appear annulated for a short distance after entering the glands.   Note the lateral branches of the intra-glandular ducts, and, on the right, an accessory lobe corresponding to a well-marked branch.

Fig. 7.   Sketch of the alimentary canal and salivary glands as they appeared when successfully dissected out.   Viewed in salt solution without a coverglass, the only addition being the schematized head in outline.   By reference to Fig. 1 the different parts will be readily distinguished.   The reservoirs contain large air-bubbles.   The posterior portion of the ventral reservoir is contracted down.   Scarcely a trace of food is contained in the stomach.

Fig. 8.   Semi-schematic cross-section through the three glands of one side in a female insect.   The central gland is situated on the left of the observer, as can be readily seen by the stellate arrangement of the secretion, which, resting on the secreting cell, runs in the form of a cone towards the central duct.   In the lateral glands the lumen is filled by darkly-staining granular secretion.

PLATE VII.

Figures 1 to 3 inclusive represent cross sections of the head of *A. maculipennis*.

Fig. 1.   Section at the commencement of the pharyngeal pump close to the pharyngeal valve.   Above are seen elevator muscles (dor. val. in Fig. 5) whose action appears to be to open the valve, by raising the dorsal plate of the chitinous tube.   Below are seen muscles in transverse section which move the maxillae.   The common salivary duct situated in the median line, its sheath not being shown.   The eye-facets at this point cover almost the entire surface of the head.

Fig. 2.   Section through the enlarged portion of the pump, cutting through the anterior portion of the posterior dorsal dilator muscles and posterior upper portion of the dorso-cephalic or "supra-oesophageal" ganglion (refer to Fig. 5).   The relaxed muscles permit the chitinous walls to form a triradiate lumen.   Above is seen the dorso-cephalic ganglion.   Attached to the upper chitinous plate are the posterior dorsal muscles which run upward and backward.   To the lateral plates are attached the lateral dilator muscles.   Below are the paired ventral nerve-cords, and outside of these the two secondary salivary ducts, and again outside of these the muscles which retract the maxillae.   tr., tracheae.

Fig. 3.   Section posterior to the preceding, avoiding the dorso-cephalic ganglion and including the posterior dilator muscles from their origin at the roof of the head to their insertion on the surface of the dorsal plates of the pharyngeal pump.

Fig. 4.   Cross-section through the contracted posterior portion of the pharyngeal pump where it joins the oesophagus.   [Small chitinous hairs protruding backward and

slightly into the lumen of the alimentary canal indicate the point at which the section is taken in the longitudinal section of the head (Fig. 5)]. The section runs somewhat obliquely, consequently there appears to be more (black) chitin on the upper side of the figure than below. The chitin here suddenly thins, a section or two further back shows a round lumen and the chitin seems to disappear, being of extreme tenuity. Viewed laterally (see Plate IX, Figs. 1 and 2), the chitin shows irregular external longitudinal ridges which correspond to the finger-like protrusions which point upward from the (black) chitinous plate in the upper part of the figure. Exceedingly fine chitinous hairs are attached to the thinning ends of the plates, and project *backward* beyond them, as seen in the lower wall of the lumen in the figure. The hairs lie in bunches in grooves formed by folds of the delicate chitinous cuticula which lines the commencement of the oesophagus. The structure is surrounded by what appears to be a ring-like sphincter muscle. The whole structure, including the muscle (measured from left to right as seen in the figure), measures but $60\,\mu$. The hairs are only visible with an immersion, by regulating the light, and focussing up and down. (Draw with the aid of Zeiss, $\frac{1}{12}$ apochromatic oil-immersion, Ocular 8.) Evidently when the sphincter contracts the chitinous hairs will serve as a sieve.

Fig. 5. Longitudinal schematic median section through the head of *A. maculipennis* (female), showing the mouth, buccal cavity, pharyngeal valve, pharynx, and commencement of the oesophagus. The dorso-cephalic and ventro-cephalic ganglia (s.g. and i.g.) are represented without detail. The labium-epipharynx (Ep. lab.) and hypopharynx (hyp.) are cut off near the base with the other mouth-parts. Note the thick chitinous lining to the pharynx and greater part of the buccal cavity, the "soft palate" with its chitinous tooth (there are two side by side), and anterior to this the chitinous spines on the thick chitinous upper "lip." The muscles figured are the elevator of the labium-epipharynx, the elevators of the palate, the muscle running to the upper part of the valve (see Fig. 1) and the anterior and posterior dorsal dilators of the pharynx. Beneath the buccal cavity runs one of the paired muscles (hy.) which works the salivary pump (s.p.), the structure of which is not represented (see Fig. 6 a). The muscular insertion covers too much of the membrane in the figure. Running backward from the pump-membrane is the common salivary duct, which bifurcates at the ventro-cephalic (infra-oesophageal) ganglion.

Fig. 6.   Transverse section of the hypopharynx, showing the salivary groove. Highly magnified.

Fig. 6 a.   Salivary pump and connected structures, as seen in a dissected specimen which has been treated with caustic potash. Viewed slightly from behind and from its ventral aspect. The common salivary duct enters (in the figure) from above, broadening where it joins the membrane, and being surrounded by chitinous spicules which serve for the attachment of muscles. In the absence of muscular action the membrane protrudes into the hollow cup, which narrows below and opens directly into the groove in the hypopharynx, which is bent to one side and cut off. The epipharynx, also cut off, is shown beneath the hypopharynx. Highly magnified. (Drawn as seen, except where the hypopharynx and the epipharynx are cut off.)

Fig. 7. Tip of the epipharynx, seen from the ventral surface. The widest portion measured externally $43\,\mu$. The structure resembles a quill-pen in general outline, the walls being of varying thickness as indicated by shading. Exceedingly minute chitinous teeth are symmetrically placed about the tip, which doubtless increase its boring power, they are borne on chitinous thickenings which offer some resemblance to an articulation. (Zeiss, apochromatic oil-immersion $\frac{1}{12}$, Ocular 8, reduced from a drawing to scale by G. H. F. N.)

PLATE VIII.

Fig. 1.　Unstained preparation of the stomach-wall of *Anopheles costalis*, showing ten oocysts of malarial parasites (par.), transverse (m.) and longitudinal muscle-bands, and a small trachea (tr.) below.　The greatest measurement of the large oocyst on the right (par.) equalled $43\,\mu$; the smallest oocyst measured $28\,\mu$.　Two of the oocysts are seen to contain sporozoites.　All the parasites were seen in a single field, the drawing representing exactly what was seen with the aid of focussing.　This represents an intense infection of the insect.　One of the authors is indebted to Professor Ronald Ross, F.R.S., for this specimen, which was prepared at Wilberforce, Sierra Leone.　(Zeiss apochromatic immersion $\frac{1}{12}$, Ocular 8.　Drawn to scale by G. H. F. N.)

Fig. 2.　Sections through the stomach wall of *Anopheles maculipennis*, infected with malarial parasites (par.) to show the position which these occupy.　Below we have the cuticula (c.) which lines the gut, next the layer of epithelial cells with their large nuclei (n.) covered externally by the elastic amorphous basement-membrane within which the muscle-bands (m.) appear to lie embedded.　(After Grassi, *Studi*, etc. 1900, Plate 2.)　The pressure exerted by the parasites is seen to deform the epithelial cells. This semi-diagrammatic figure shows what we have seen if we except the parasites.

PLATE IX.

Figs. 1 and 2 represent chitinous parts only, owing to treatment with caustic potash. They represent the pharyngeal pumps of a female (Fig. 1) and male (Fig. 2) *Anopheles maculipennis in situ*, parts of the exoskeleton of the head, which would impede the view, having been dissected away.　The pump is seen to be much larger in the female, as well as more highly chitinized (darker in the photograph).　The dorsal flexure of the tubular portion of the pharynx is well-marked in Fig. 2.　Some of the delicate chitin lining the oesophagus is seen in Fig. 1 attached to the posterior portion of the pharynx, the very transparent tube being scarcely discernible and folded upon itself.　In both figures the ends of the bulbs are seen to bear delicate ridges, regarding which see description of Fig. 4, Plate VII.　In Fig. 1, the situation of the pharyngeal valve is indicated by a small black mass on the top of the chitinous tube leading to the right of the figure, the direction of the tube, corresponding to that of the buccal cavity, also changes at this point.　In Fig. 2, above and on the left is seen the clypeus, and beneath it the mouth appendages, including the labium which is covered by scales and hairs.　The photographs were both taken to the same scale.　Our thanks are due to Mr Walter Mitchell, attendant in the Pathological Laboratory, for the pains he has taken in their reproduction.

Fig. 3.　Transverse section through the central salivary gland of *A. maculipennis*.　On the right, running up and down is a mass of muscle.　Above, a small part of the darkly staining (granular type) lateral (here dorsal) gland is seen.　Below the central gland the presence of a large trachea is faintly indicated in cross section.　Situated about the periphery of the gland, which is indented, one sees the darkly staining nuclei of the salivary cells.　In the centre lies the intra-glandular duct, which together with the secretion of the cells contains innumerable small bodies, which in a few cases are seen to be elongated.　These are sporozoites, the insect having been infected with the parasites of aestivo-autumnal fever.　One of the authors is indebted to Professor Grassi for this specimen, which was given to him some three years ago, and it will be seen that it almost exactly reproduces a very beautiful drawing given by Grassi (Plate II, Fig. 19) in the first and second editions of his *Studi* (1900).　The microphotograph ($\times 500$) is the excellent work of Professor Carl Günther, of the Hygienic Institute, Berlin.
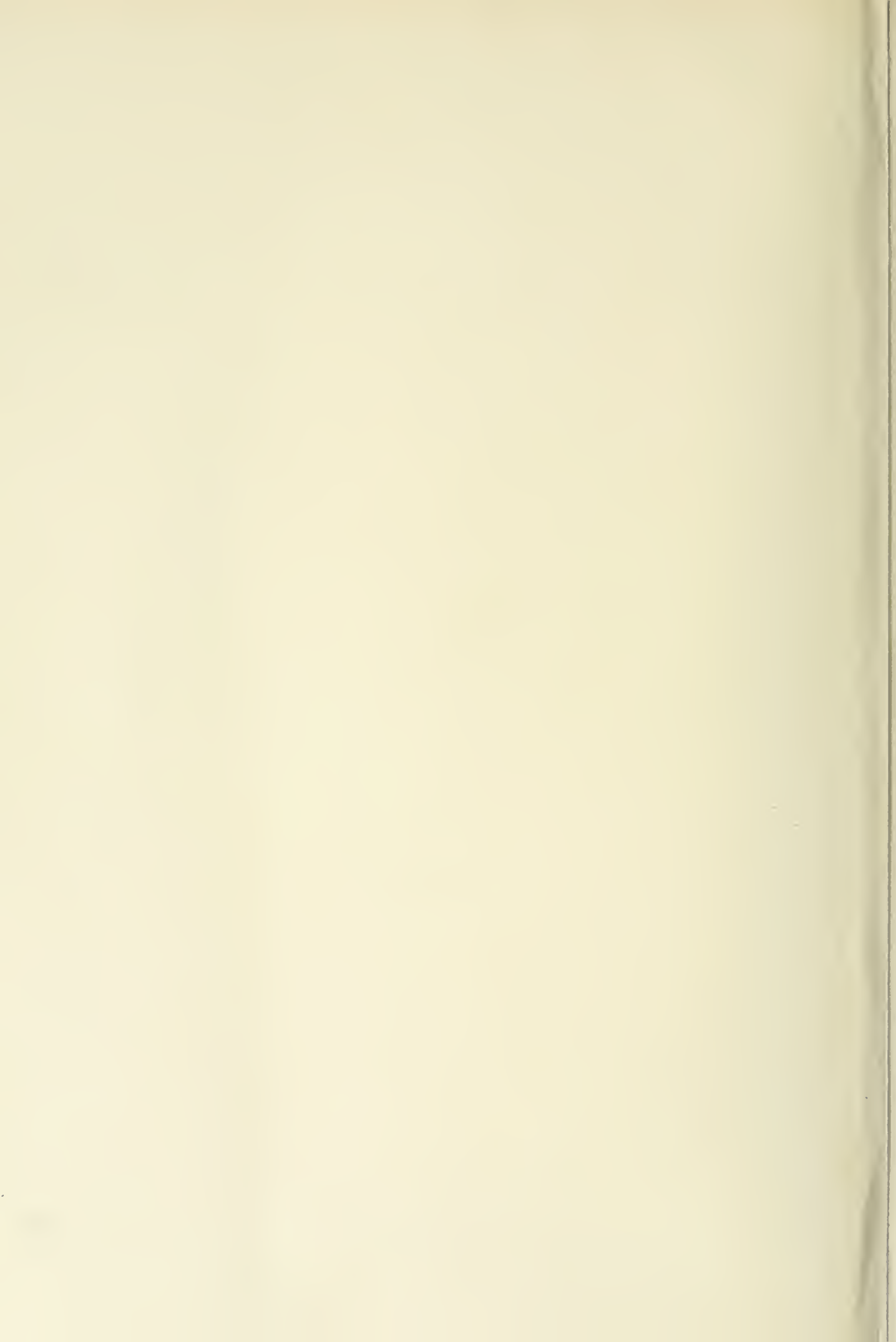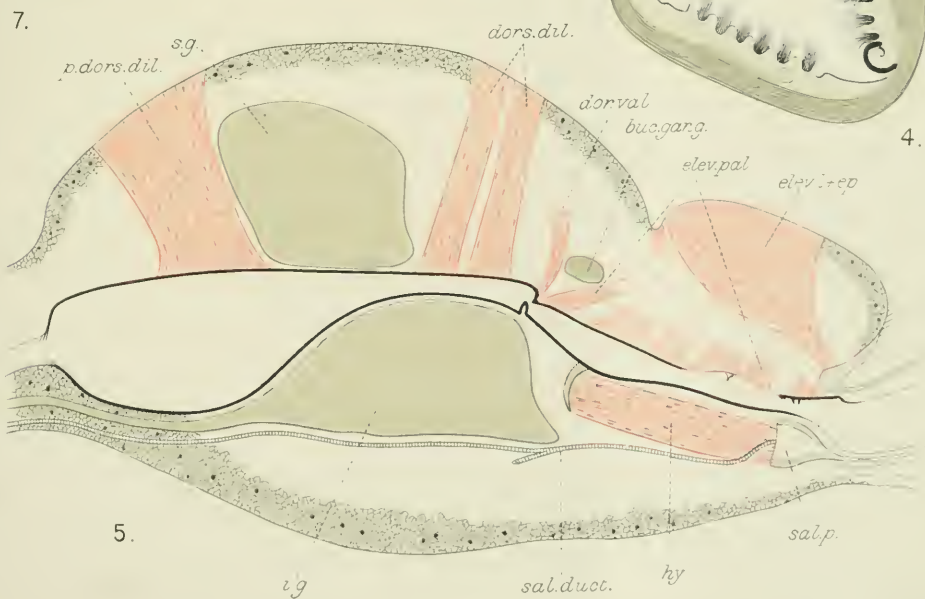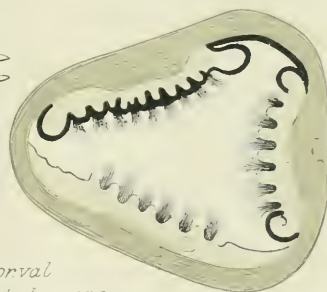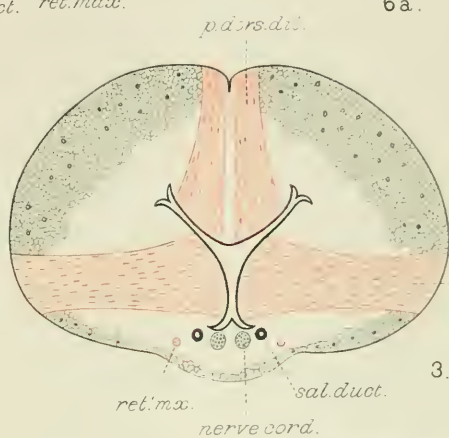
buccal cavity

pharynx.

salivary reservoirs.

sal. duct

oesoph.

sal. glands

oesoph. valve
and caeca

midgut begins

ventral
reservoir

rectal papilla

"stomach"

malpighian tube

midgut ends

ileum

colon

rectum

anus

p

1.

2.

3.

4.

5.

PLATE 6.

8.

7.

6.

E. Wilson, Cambridge

PLATE 7

200 b

6.

mus of the valve

op.gang

ch.t.

pro.max.

sai.duct. ret.max.

1.

6a.

s.g.

p.dors.dil.

tr.

lat.dil.

ret'mx.

nerve cord

sal.duct.

2.

p.dors.dil.

lat.dil.

ret'mx.

sal.duct.

nerve cord.

3.

7.

4.

p.dors.dil.

s.g.

dors.dil.

dorval
buc.gar.g.

elev.pal

elev.l.ep

Ep.lab

hyp.

i.g

sal.duct.

hy

sal.p.

5.

E. Wilson, Cambridge

Fig. 1.

Fig. 2.

Fig. 3.

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APPENDIX.

Bibliography of recent Literature on Malaria, and relating chiefly to Prophylaxis, Epidemiology, and Mosquitoes,

by G. H. F. N.

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THE DISTRIBUTION OF THE DIPHTHERIA BACILLUS AND THE BACILLUS OF HOFMANN IN THE THROATS OF "CONTACTS" AND NORMAL PERSONS.

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ATTEMPTS have been now made in various towns, schools, and institutions to stamp out epidemics of diphtheria by isolating the sick until their throats have been proved by bacteriological examination to be free from the diphtheria bacilli.

In several of these cases, acting on the well-known fact that persons who have come in contact with the diseased may acquire and carry in their throats and noses virulent diphtheria bacilli without themselves being ill, "contacts" have been examined, and those found to be harbouring diphtheria bacilli isolated in the same manner as the convalescents.

These efforts to check the spread of the disease have for the most part been based on the assumption that virulent diphtheria bacilli do not occur in the mouths and noses of persons who have not been in some way exposed to persons suffering from the disease, or persons who have acquired the bacillus by being so exposed. This view is not however held by all the authorities on the subject; for while many are of the opinion that virulent diphtheria bacilli are never to be found in the throats of healthy persons, who have had no opportunity of acquiring the bacillus by contact, others believe that it does occur in small numbers amongst the normal population.

Should the view of the latter school be accepted the method of attempting to suppress epidemics by the isolation of healthy individuals is not only likely to prove useless, but entails unnecessary hardships on the isolated persons.

It is with the purpose of showing that virulent diphtheria bacilli do not exist in the noses and throats of healthy persons, who have had no opportunity of acquiring them by contact, and that the method of isolating convalescents and healthy persons harbouring the bacillus is consequently likely to be of great benefit in suppressing epidemics of the disease that the following statistics have been brought together.

The Views of various Authorities, and the Statistics on which they are based.

The Occurrence of Diphtheria Bacilli in notified cases.

Novy[71] gives a table showing the results of observations by European workers between 1886 and 1895, in which 2,846 cases of diphtheria were examined and diphtheria bacilli found 2,344 times (82·4 %).

European and American observers combined, examined 8,186 cases, finding the specific bacillus in 5,943 or 72·6 per cent.

French investigators in the Institut Pasteur obtained the bacillus in 701 out of 960 cases (73 %).

During 1894 certain German workers satisfied themselves of the presence of the bacillus in 945 out of 972 cases, giving a percentage of 97·2.

The work of Park and Morse[79] showed that out of 5,340 suspected cases, 67·5 % were true diphtheria.

Woodhead[101] states that of the 12,172 cases admitted into the Metropolitan Asylums Board Hospitals during 1895–6, and certified as suffering from diphtheria, at least 20 %, or about 3000, offered no bacteriological evidence of diphtherial infection.

Cobbett[18, 20] in two outbreaks at Cambridge, in 1900 and 1901, found diphtheria bacilli in 57 % (42 cases), and 66·6 % (27 cases) of notified cases. Except in one of the negative cases two or more subsequent examinations made by him confirmed the original diagnosis.

At Colchester[41] diphtheria bacilli were found in 87 % of notified cases. Repeated examinations were made of the negative cases.

The results of nearly 27,000 certified cases quoted above show that there was bacteriological evidence of diphtheria in about 72 %.

The Occurrence of Diphtheria Bacilli in persons who have been in contact with cases of the disease, or with others who acquired the bacillus in this way.

All observers are agreed that virulent and dangerous diphtheria bacilli occur in the mouths of certain healthy persons who have come in contact with the sick, or with others who, like themselves, harbour diphtheria bacilli.

The proportion of infected to non-infected contacts is subject to great variation according to the investigations of different observers. To some extent these differences probably depend on the measures taken to promptly isolate the sick, the class of persons examined, and the views of the observer as to the importance of the bacilli which he finds.

Families.   Cobbett[19] found every member of one family to have diphtheria bacilli in their throats.   From three the organisms were isolated and found to be virulent (100 %).

Spirig[88] examined the children of two families numbering four and six respectively.   There was one case of clinical diphtheria—of the remaining nine children six were found to harbour diphtheria bacilli, one Hofmann's bacillus, and two no bacilli; out of the infected six five subsequently developed diphtheria.   Infected contacts 66·6 per cent.

Park and Beebe[78] amongst 48 children from 14 infected families found bacilli in 50%.   Of six cultures tested all were virulent.

Schools.   Goadby[38] examined a school with 600 children—21 cases of diphtheria had previously occurred.   He found diphtheria bacilli present in 190 cases (34·1 %).   The morphology in culture was alone relied on.

Berry and Washbourn[8] out of 142 girls examined in a school, in which several cases of diphtheria had occurred, discovered diphtheria bacilli in 17 (11·9 %).

Amongst 200 scholars in a truant-school Denny[29] found 22 with diphtheria bacilli shortly after four cases of true diphtheria had occurred (11 %).

Hospital Wards.   Chatin and Lesieur[17] made observations on 75 children in an asylum in which there had been one case of diphtheria. 14 of the children were suffering from sore throats, of whom two had diphtheria bacilli; the remaining 61 were free—(2·66 %).

Park and Beebe[78] found 6 out of 55 children in a foundling hospital

to harbour diphtheria bacilli. Of these 5 were virulent. Some cases of diphtheria had from time to time occurred in this institution (virulent bacilli 9 %).

Lister [61] in the Shadwell Hospital examined 125 children, 69 of whom had nasal discharges, and found 61 (24 with discharges) had diphtheria bacilli (48 %).

Müller [70] made observations on 100 children in a general ward in the Charité in Berlin. Six had diphtheria bacilli without illness on admission. Two days later bacilli were found in 14 others (20 %).

General Contacts. Kober [56] at Breslau showed that 15 out of 128 general contacts harboured diphtheria bacilli (11·7 %).

Meade Bolton [67] stated that of 214 healthy persons examined who had previously been exposed to infection 45·5 % showed diphtheria bacilli.

Cobbett [19] during an outbreak at Cambridge in 1900, on examining 650 persons, mostly school-children, found 19 (2·9 %) affected with diphtheria bacilli. Experiments on animals showed that out of nine pure cultures tested, three were not pathogenic to guinea-pigs.

In the Colchester [41] epidemic I found 54 persons (10·4 %) harboured diphtheria bacilli out of 519 examined. All these were school-children or persons connected with schools. Morphological and cultural methods were relied on as no tests for pathogenicity could be undertaken there.

Contacts with Sore Throats.

Goadby [38] noted in his cases that out of the 586 examined 262 had enlarged tonsils, and 196 abnormal throats. 34·1 % harboured diphtheria bacilli. Pakes [74] examined 3,000 cases of sore throat, finding diphtheria bacilli in 343 (14·3 %).

The mean of the examinations of the very close contacts in families gives 51 % of infected persons; in more distant contacts in institutions 40 %, and in schools 24 %; whilst in the class of general contacts only 12 % harbour diphtheria bacilli.

The results of the examination of healthy persons who have not recently been in contact with the disease.

Kober [56] examined 600 healthy school-children and discovered diphtheria bacilli in 15 cases. The bacilli from 10 were however found to be non-pathogenic. Of the five children with virulent bacilli one

sat at school next a child who had diphtheria eight weeks before, three were playmates of neighbours' children who had had diphtheria recently, and the fifth had associated with a family in which a fatal case of the same disease had occurred 10 weeks before. Five of those with non-virulent bacilli were proved also to be old contacts (non-contacts with virulent diphtheria bacilli 0 %).

Garratt and Washbourn [37] examined 666 cases of scarlet-fever admitted under their care at the London Fever Hospital, and found in 8 (1·2 %) cases bacilli morphologically identical with the diphtheria bacillus; their examinations were conducted on a class of persons especially prone to acquire the diphtheria bacillus if brought in contact with the disease. The virulence of the organisms was not tested.

Denny [29] of Brooklyn, Mass., examined 235 healthy individuals (216 children and 19 adults), a large proportion of the well-to-do class. He only once, in a school-girl, found the diphtheria bacillus. So far as was known the girl had not been in contact with a case of diphtheria. The bacilli were so few that a pure culture could not be obtained (·4 %).

Park and Beebe [77] examined 330 persons, chiefly hospital patients. Diphtheria bacilli were obtained in culture from 32 persons, but 24 cultures proved to be non-virulent. The presence of all but two of the eight virulent examples was accounted for by recent contact, and these two occurred in adults. (Virulent bacilli unaccounted for by contact in ·6 % of these persons.)

Goadby [38] examined 100 school-children from a school in which there had been no diphtheria for two years and found 18 with diphtheria bacilli. This school was examined as a control to his previous experiment in which diphtheria was present (see p. 218). The disease was therefore prevalent in the neighbourhood and the high percentage of diphtheria (18 %) is to some extent accounted for. Morphology was alone relied on, and virulence was not tested.

Herman Biggs [9] examined 330 healthy persons and found virulent diphtheria bacilli in eight, and non-virulent acid-producing bacilli in 24 (2·4 % of virulent bacilli).

The Committee [65] of the Massachusetts Association of Boards of Health feel justified in the inference that in urban communities at least 1 to 2 % of well persons amongst the general public are infected with diphtheria bacilli; but their experiments show that only 17 % of these bacilli are virulent. In other words that 17 out of 5,000 to 10,000 of all persons harbour diphtheria bacilli which are dangerous to public health.

Their figures[1] show however that in the following places, Ontario, Newton, Springfield, Washington, Lowell, Waltham, and Providence, in which 50, 63, 185, 221, 250, 297, and 927 persons respectively were examined, the typical bacilli were only found as follows, 0, 0, 0, 2, 2, 2, 4, namely 10 cultures with typical bacilli in 1993 persons ($\cdot5$ %). If only 17 % of these are virulent the percentage of virulent bacilli in the places mentioned is only $\cdot085$.

In New York, Brooklyn, and Minnesota on the other hand, where it is stated that diphtheria was prevalent at the time of these investigations, $3\cdot66$ %, $2\cdot32$ %, and $2\cdot89$ % of persons harbouring diphtheria bacilli were found amongst 82,129 and 4,250 persons respectively; whether or not diphtheria was present in Boston is not mentioned, but in this city 27 infected persons were found out of 892 examined, giving a percentage of $3\cdot02$.

Pugh [81], working in the North-Eastern Fever Hospital, has come to the conclusion that "in large centres of population, where diphtheria always exists, diphtheria bacilli are to be found in a not inconsiderable proportion of school-children. In the absence both of the evidence of clinical diphtheria and of a history of exposure to that affection, the bacilli are, in the majority of cases, of a non-virulent or saprophytic type and of little hygienic importance; in cases on the other hand, where the clinical supports the bacteriological examination the bacilli are almost certainly virulent, and therefore dangerous: while in cases where the patient is known to have been exposed to infection the chances are great that the organisms are of the pathogenic variety, and such cases should always be regarded with grave suspicion."

Hewlett and Murray [46] investigated the throats of all children (385) admitted into the Victoria Hospital. (The cultures were examined at the Jenner Institute of Preventive Medicine.) They found diphtheria bacilli in 58, or 15 %—only three cultures were examined for virulence and of these two were stated to be only slightly, if at all, virulent.

Except in these three morphology was alone relied on.

As a result of their examinations Hewlett and Murray argue that 15 %, or one out of every seven, of normal children amongst the general community have diphtheria bacilli in their throats. They say "the morals deducible from these figures are almost too obvious to need detailed statement: it is clear that babies and young children are

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[1] In their table the authors of this report include Boston, New York, and Brooklyn, with the places quoted in one set. I have here separated the three places just mentioned for reasons given in the text.

not the innocent and harmless creatures usually imagined, and that kissing and other similar signs of artificial demonstration of childish affection should be discouraged."

Their results are entirely at variance with the other observations which have been quoted.

Although these authors do not as far as I am aware draw from their experiences the inference that the isolation of infected contacts can be of little importance, scarcely any other construction can be placed upon their observations. Moreover they seem to have made no inquiries into the antecedents of their patients and do not state whether diphtheria was prevalent, or not, at the time.

The following table shows the prevalence of diphtheria bacilli amongst normal persons as ascertained by investigations in which no inquiries as to contact appear to have been instituted.

Observer	Persons examined	Organisms morphologically resembling diphtheria bacilli	Virulent diphtheria bacilli	Non-virulent diphtheria bacilli
Massachusetts Committee [65]	1993	10	—	—
Garratt and Washbourn [37]	666	8	—	—
Hewlett and Murray [46]	385	58	1?	2
Herman Biggs [9]	330	32	8	24
	3374	108 (3·2 %)	9 (·2 %)	26 (·7 %)

It is very noticeable in such statistics that whenever the virulence of the organisms discovered has been tested a large proportion have been found to be non-pathogenic. In the above list 26 out of the 35 (74 %) tested turned out to be devoid of virulence.

The following table, on the other hand, shows the results of observations on normal throats in which careful inquiries were instituted as to the possibility of recent infection.

Observer	Persons examined	Virulent diphtheria bacilli	Non-virulent diphtheria bacilli	
Kober [56] 1	590	0	5	
Park and Beebe [77]	324	2	24	
Denny [29]	235	1	0	Virulence not tested
	1149	3 (·26 %)	29 (2·5 %)	

The various figures which have been quoted merely emphasise the well-known facts that diphtheria bacilli are present in the large majority of cases which have been diagnosed as diphtheria on clinical

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1 Persons found on inquiry to be "contacts" have been excluded in this table.

grounds alone, and that contacts with clinical cases, as well as persons who have been associated only with the latter, are liable to become infected with diphtheria bacilli. The proportion of infected persons depends, as has been shown, on their relationship to the clinical cases.

On the other hand, amongst persons who have had no opportunity, as far as can be ascertained, of acquiring the bacilli from clinical cases, or "contacts," virulent bacilli are present in very small numbers.

Some investigators have apparently relied entirely on the morphological characters of the bacilli, but others have not only cultivated them but have tested their pathogenic power. The results of the latter are naturally the most trustworthy. They have shown that in a large proportion of their cases in which bacilli morphologically and culturally identical with diphtheria bacilli have been found they were devoid of virulence. On further inquiry amongst those who harboured virulent bacilli they have elicited the fact that in reality they were, in almost every case, recent contacts. After eliminating all such recent contacts it is found that virulent diphtheria bacilli occurred, and could not be satisfactorily accounted for, in two out of 1149 persons. In a third person bacilli morphologically identical with diphtheria bacilli were discovered, but were too few in number to allow of a pure culture being obtained.

I think it may therefore be safely assumed in the absence of conclusive evidence to the contrary that virulent diphtheria bacilli are seldom if ever present in the throats of healthy persons, who have not been in contact with cases of diphtheria, or infected contacts.

The Results of Isolation.

The attempts which have been made at Cambridge [18–20] and Colchester [41] to stamp out diphtheria epidemics by isolation have been attended by encouraging results. The measures employed depended on the assumption that virulent diphtheria bacilli do not occur in the mouths of healthy persons (non-contacts). The methods by which suitable persons were examined and isolated are given at length in the papers mentioned, but the main points may be recapitulated here.

(1) As far as possible all notified cases were examined, and isolated until three consecutive negative examinations showed them to be free from diphtheria bacilli.

(2) As far as possible all cases of sore throat brought to the notice

of the medical practitioners, and more especially those occurring amongst school-children, were investigated.

(3)   As far as possible all children belonging to the families in which diphtheria cases had occurred, and all persons known, or likely, to have been in contact with them, were also examined, and if diphtheria bacilli were found in their throats, isolated.   Particular attention was paid to school contacts.

(4)   The means by which the disease is generally considered to be communicated to others by patients and contacts were explained to the school teachers, and precautions taken to guard against its spread by infected articles.

(5)   The administration of antitoxin as a prophylactic to healthy contacts, who showed the bacillus in their throats, was encouraged, as was also the use of antiseptic mouth-washes.

At Cambridge [18] in 1900 the results of these measures were highly satisfactory, and their application in the following spring [20] was again successful.

At Colchester [41] the most striking results were obtained in checking the progress of the disease in the schools.   These are given in tabular form in the paper referred to, but the general results may I think be again stated here with advantage.   Cases of diphtheria were of constant occurrence amongst the scholars until all known healthy contacts had been carefully examined and those harbouring diphtheria bacilli excluded from school.   On the reopening of the schools after the completion of the precautionary measures no case of diphtheria was notified amongst the scholars except in three out of 19 schools.

In the first of these three schools eight weeks elapsed, then three cases were notified.   (One died before any cultivation could be made, and in the other two no bacilli could be found.)   In the second school two cases occurred after a lapse of four weeks; but in the third there was a small outbreak extending over four weeks.   All the scholars (112) in the infected portion of this school were examined, and five harbouring diphtheria bacilli were found, and excluded.   Subsequently no further cases were notified[1].

In   this   outbreak   the   administration   of   antitoxin   without bacteriological examination was not encouraged, as persons thus

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[1] The cases mentioned are the only ones which occurred amongst the school-children during the 10 weeks which followed the opening of the schools.   After that period I was no longer in a position to carry on the record.

rendered immune carry the organisms in their mouth for long periods and may act as unknown centres of infection[1].

Berry[8] gives the results of isolating infected children in a girls' school with 200 scholars. Diphtheria was introduced (Feb. 25th) after the Christmas holidays. A series of mild sore throats followed till March 27th, when another case of the disease occurred, together with three examples of sore throat associated with the diphtheria bacillus. These persons were isolated. A further series of sore throats however continued till April 30th, when five cases of diphtheria were notified. Washbourn then examined all scholars with any abnormality of the throat (142), and discovered diphtheria bacilli in 17 of them. All the latter were isolated and not allowed to return to school till they had been declared free. No further cases of diphtheria, or sore throat, occurred.

A good example of the advantages of efficient isolation is given by Burnett[14], and Peck[80] cites the case of a school in which the effect of isolation was for a time very beneficial. An abstract of these cases is given later (p. 239).

Goadby[38] found some benefit resulted from partial isolation in a school, although the system eventually broke down.

In Providence[65] the method of keeping at home the infected members of families in which diphtheria exists was carried out faithfully for a period of five years, but has been abandoned because it met with a very decided opposition from both the laity and medical profession. It should be noted that in Providence only one negative culture-test was required for release.

Wesbrook, Wilson, and McDaniel[97] attempted to stamp out diphtheria in a school by the isolation of the infected children. Although their method of isolation eventually broke down they assert that " the effects of the isolation, and the thorough looking over to which the children were subjected, as well as the local treatment of the throat and nose in ridding them of diphtheria bacilli had an apparent beneficial effect on the general health of the school."

The examples which have just been cited clearly demonstrate the advantages to be derived from thorough examination, and isolation, whenever practicable. Even in those cases in which isolation was not

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[1] Instances of persons thus temporarily rendered immune acquiring the disease without further contact are given by Jump[52], and experimental observations on this subject have been made by Bullock[13].

efficiently carried out the observers considered that some good effects followed.

Cobbett [21] considers that " the duty of discovering, isolating, and disinfecting the former class of persons (infected with virulent bacilli) is becoming more and more the urgent duty of the sanitary authorities. For the fact that they are not scattered broadcast throughout the community as was once supposed but are confined to the class of persons whom we conveniently term 'contacts' renders their discovery a practical possibility, and offers a fair prospect that at least the great majority of them may in the near future be subjected to isolation and antiseptic treatment with immense advantage to the public health."

By no means all observers are however in complete agreement with this opinion. Several investigators, while acknowledging the danger of infection to others through healthy infected contacts, at the same time do not agree as to the practicability of their isolation.

The Massachusetts Committee [65] arrived at the following conclusions. "There are scattered among the general public a considerable number of persons, not recently and directly exposed to the disease of diphtheria, who have typical diphtheria bacilli in their throats." They compute that there would be 8000 such in Boston, and continue, "the mere statement of this fact shows how entirely futile it is to attempt to seek out and isolate the whole of this number. If this cannot be done it is useless and unjust to isolate the small number that it may be possible to discover."

They advise however that children in infected families should be kept away from school and public places; that teachers, nurses, and others who are brought in close contact with children, as well as milk-men, should not be allowed to continue their work if found to be harbouring diphtheria bacilli.

They also express the opinion that in schools and institutions, if the infection is not too wide-spread, infected persons, whether sick or well, should be isolated until free from bacilli.

Further, they consider that when diphtheria appears in a community which has been for some time free from it, it is advisable to isolate all persons who have been brought in contact with the patient until it should have been shown that they are free from diphtheria bacilli. Finally, they advise that the dangers of infection should be pointed out to the infected individuals, or their guardians, as well as to school

teachers, and means indicated for minimising the danger of infection by articles used by patients or contacts[1].

Prof. Wesbrook, one of the signatories to the report, modifies his assent as follows. "It may sometimes be impracticable to isolate from the public all the well persons in infected families, schools or institutions, though it should be done as a routine if at all possible."

Welch[96] in summarizing the results of work on this subject up to 1894 remarks, "all members of an infected household should be regarded as under suspicion, and, where isolation is not enforced, the healthy as well as the sick should be prevented from mingling with others until cultures, or a sufficient lapse of time, give the presumption that they are not carriers of infection."

From these quotations it is seen that while some authorities consider that all infected persons harbouring virulent bacilli should be isolated, others think that this should only be done under special circumstances, and others again would merely isolate the infected members of families in which diphtheria exists.

On the other hand, it would certainly appear from the deductions of certain bacteriologists as to the frequency of diphtheria bacilli in normal throats, that they regard isolation as of little value.

If it be admitted then in accordance with the great bulk of expert opinion, that the isolation of infected persons is a necessary measure for checking outbreaks in schools, institutions, and towns, the following questions have to be considered in detail by the authorities. Which of the various types of bacilli should be considered dangerous? How many consecutive negative examinations are necessary before infected persons, whether convalescents or healthy contacts, can be released from isolation? How long are diphtheria bacilli likely to persist in the throats of these persons? How are the bacilli communicated by infected persons to others? What classes of persons should be examined, and, if found infected, isolated? Can infection be carried otherwise than through infected persons?

Which of the various types of bacilli should be considered dangerous?

The divergent views held on this question even at the present day are well illustrated in the Report of the Massachusetts Committee[65].

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[1] This report is signed by C. V. Chapin, H. W. Hill, S. W. Abbott, F. H. Baker, F. P. Denny, E. P. Gorham, W. H. Gore, A. Hudson, T. B. Shea, Theobald Smith, and F. F. Wesbrook.

The several collaborators were requested to detail the various bacilli they found according to Wesbrook's [08] types, and also to state in each case whether or not a positive diagnosis of the presence of diphtheria bacilli had been made.

In Providence, on the basis of the Committee's belief that A, C and D of Wesbrook's types should be considered chiefly, or solely important, there would be ·43 % of positives. If all granular or barred forms, but not solid forms, be included, as Prof. Gorham of Providence states, there would be 3 % of positives. If all be included there would be about 25 %. The number actually reported positive makes about 9 %.

In Washington the positives formed ·9 % on the Committee's standard; but 22 % were actually reported positive. In Boston on the Committee's standard 3·02 % were positive; but only 1 % was so reported.

It is evident from the above that some standard must be adopted in dealing with an outbreak.

Cobbett [19] has carefully worked out the virulence of the various types of bacilli met with during the epidemic of 1900 at Cambridge. A standard based on these observations was adopted by him in the outbreaks of 1900 and 1901 at Cambridge, and by myself at Colchester [41].

He recognises five morphological types of diphtheria bacilli from young serum cultures[1]:

    (1)   Oval bacilli with one unstained septum, very young forms[2].
    (2)   Long, faintly stained, irregularly beaded bacilli.
    (3)   Regularly beaded bacilli, streptococcal forms.
    (4)   Segmented bacilli.
    (5)   Uniformly stained bacilli.

The majority showed polar bodies by Neisser's method of staining in cultures less than 24 hours old, grown at 37° C., and all formed acid in 48 hours when grown in glucose broth (1 %) in pure culture.

Cultures grown in broth for 48 hours were injected subcutaneously into guinea-pigs of 200—500 g. in doses of ·1 c.c. In such doses 25 virulent bacilli killed within three days. In four examples of non-virulent diphtheria bacilli doses of 2 c.c. did not cause death. Cobbett found no intermediate degrees of virulence amongst diphtheria bacilli.

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[1] The medium used for growing the cultures was alkaline ox serum, to which 1 % of glucose had been added. First proposed by Prof. Lorrain Smith, of Belfast. *Brit. Med. Journ.* Vol. II. 1894.

[2] The author does not mean to imply that in any pure cultures these would at any time be the only forms met with. Certainly in my experience this has never been the case.

At Colchester [41] many pure cultures were isolated in the following way. The top of a suspicious colony was touched with the point of a sterile platinum needle. The needle was then transferred to a tube of sterile salt solution ($\cdot7\,^{0}/_{0}$) and, after its removal from the fluid, a coverslip preparation was immediately made with it. If the latter showed by microscopic examination the desired organisms and no others, a serum subculture was sown from the salt solution tube. In some cases a second subculture from the first was necessary before a pure culture could be obtained.

All organisms which were considered on morphological grounds alone to be diphtheria bacilli, and which were isolated and tested, were found to form acid in glucose broth. Nearly all stained well by Neisser's method and in young subcultures retained the appearance of diphtheria bacilli. At the time there was no means of carrying the investigation further and testing their pathogenic properties.

Almost every original cultivation, in which organisms resembling diphtheria or Hofmann's bacilli occurred, was stained by Neisser's method, or Cobbett's modification [21] of it, and, except in very few instances, it was found that diphtheria bacilli showed polar bodies, whereas Hofmann's bacilli did not—about 2000 cultures were examined.

Cobbett [19] observes that " the bacillus of Hofmann in young serum cultures appears with considerable regularity as a darkly staining oval bacillus of somewhat variable length, with one narrow, unstained septum. These bacilli present a very characteristic appearance and do not at all closely resemble the common adult forms of the true diphtheria bacillus. Occasionally, however, colonies are met with which contain a fair number of bacilli with several septa, and then the diagnosis is more difficult." He found that they showed no polar bodies by Neisser's method. Of 69 pure cultures of this organism which he isolated, and tested, not one produced acid in glucose broth or caused any local oedema in guinea-pigs. He finally says that " once one had become well acquainted with the range in its variation it was fairly easy to recognise the diphtheria bacillus and distinguish it from all others"; but goes on to remark " that the eye cannot become sufficiently trained for this purpose unless the observer frequently tests the opinions he forms on morphological grounds, by isolating his cultures and testing them in various ways, including the injection of animals." No short Hofmann-like virulent bacilli such as have been described by Wesbrook were encountered by him.

I have, however, met with colonies of segmented bacilli in which

very few, if any, of the small typical Hofmann forms occurred.   These organisms are clubbed, but broader and take the stain more deeply than diphtheria bacilli.   The stained segments are very dark and the septa narrow and well defined, running in all cases transversely across the bacillus.   They do not show any polar bodies by Neisser's method of staining, and in the few cases (5) I have examined are non-patho-genic to guinea-pigs, and do not form acid in glucose broth.   Moreover in subculture they revert to the typical short form of Hofmann's bacillus with an occasional long specimen.   It is only after several days' growth in subculture that many long, segmented, forms again become visible. These I term the pseudo-diphtheria form of Hofmann's bacillus (see Plate 10).

The bacilli isolated and tested by Cobbett [19] and myself may be classified as follows [1]:

I.   Bacilli identical in appearance both in culture and under the microscope with diphtheria bacilli :

a.   Pathogenic acid-producers, usually showing polar bodies by Neisser's method = virulent Klebs-Löffler bacilli.

b.   Non-pathogenic acid-producers.   Neisser's staining generally positive (?) = so-called attenuated diphtheria bacilli.

II.   Bacilli somewhat resembling diphtheria bacilli but (generally shorter and) stouter :

a.   Non-pathogenic, non-acid-producing segmented bacilli, showing no polar bodies by Neisser's method = pseudo-diphtheria type of Hofmann's bacillus.

b.   Non-pathogenic, non-acid-producing short bacilli.   No polar bodies by Neisser = typical Hofmann's bacillus.

The question whether the pseudo-diphtheria or Hofmann's bacillus is an attenuated diphtheria bacillus, capable of becoming dangerous, has been considered by various authors [19].

Roux and Yersin [85] thought they could increase the virulence of a bacillus which caused oedema but did not kill, but were unable to give virulence to a non-virulent form.

Hewlett and Knight [45] in 1897 considered that by heating they once converted a diphtheria into a pseudo-diphtheria bacillus, but were unable to repeat this, stating that "the amount of heating

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[1] Founded on the classification first introduced by Park and Beebe, New York, *Med. Rec.* xlvi. 1894.   Cited by Cobbett.   The table is not quite the same as that given by the latter, as I have inserted (ii) A and the results of Neisser's method of staining.

Fig. 1.   Hofmann's Bacillus (Pseudo-diphtheria type)
24 hour culture on serum at 37 C.

Fig. 2.   The same as Fig. 1, subculture 24 hours old on serum.   This
represents the typical Hofmann's Bacillus.   (Both figures drawn
with aid of camera lucida, stained with Löffler's methylene-blue
(diluted 1 : 5).   Zeiss $\frac{1}{12}$ im., No. 4 oc.)

required is a very delicate factor; too little leaves the bacilli comparatively unaltered, a little more kills them completely." They also thought that "in one or two cases" they had succeeded in transforming the pseudo into the diphtheria bacillus. They started with a typical pseudo "from the throat of a nurse who had been nursing a case of diphtheria," and cultivated it in agar for 19 generations. During this time they state it constantly altered its form, "some subcultures showing typical Klebs-Löffler forms, others typical pseudo." The 19th and 20th generations were cultivated on serum for a week, and from the latter "a broth culture was made and incubated for a week." For no better reason than this proceeding the organism suddenly became virulent. They discuss, but reject, the possibility of having started with a mixture, and on this evidence finally state: "we therefore consider that the pseudo is sometimes a modified Klebs-Löffler, though perhaps not always, as possibly more than one species having the same morphology may exist."

In 1898 Richmond and Salter [83] briefly stated that by repeated passages through certain birds they had been able to convert Hofmann's into diphtheria bacilli, but gave no details. These were supplied for one case by Salter [86] in the following year. "The bacillus employed was originally obtained from a case of post-scarlatinal diphtheria." It was a typical Hofmann morphologically and was non-virulent. Its reaction in a sugar medium, one of the essential characters of the diphtheria bacillus, does not appear to have been tested. After two passages through goldfinches it was said to present a transitional appearance between a pseudo-diphtheria bacillus and a short Klebs-Löffler. After four such passages it produced oedema, but not death, in a guinea-pig, but after the fifth passage 5 c.c. killed a guinea-pig in four days with all the symptoms of experimental diphtheria. It now formed acid in neutral broth and its action was neutralized by antitoxin.

In 1902 Ohlmacher [72] published some observations on this question. He experimented with three organisms, and concluded that by a short sojourn in an immune animal a diphtheria may be converted into a pseudo-diphtheria bacillus, and that the reverse may be brought about by passing the organism through a susceptible animal. His experiments, however, only show that a long granular diphtheria bacillus after recovery from the subcutaneous tissue of a rat became short and uniformly staining, but still formed acid in glucose media. A uniformly staining, but pathogenic, bacillus after recovery from the spleen of a guinea-pig became granular, and a short uniformly staining, and slightly virulent

bacillus (killing in 7 days) after its passage through an animal became long and granular and more virulent.

Lesieur [59] could agglutinate by the serum of horses immunised by cultures of diphtheria bacilli certain varieties of this organism but not others. Hofmann's bacillus behaved in the same manner. He considers that the fact constitutes a new presumption in favour of the identity of certain species of pseudo-diphtheria bacilli with the true diphtheria bacilli.

On the other hand Lubowski [63] working with non-virulent diphtheria bacilli succeeded in immunising animals and producing a serum which agglutinated not only these bacilli but also 23 different races of quite typical diphtheria bacilli. The antiserum had no action on pseudo-diphtheria bacilli.

"In view of the wide distribution of Hofmann's bacillus among healthy persons in Cambridge and elsewhere the conclusion arrived at by Richmond and Salter that the pseudo-diphtheria bacillus is an attenuated variety of the true causal agent of diphtheria is, if well founded, of great importance. But until the position of the bacillus of Hofmann has been clearly established and it has been proved capable of being converted into the virulent diphtheria bacillus, not merely by laboratory procedures, but further under natural conditions, we must not conclude that the causal agent of diphtheria is wide spread" (Cobbett [19]).

There is no evidence that bad drains and insanitary environment can ever convert non-virulent into virulent bacilli or originate diphtheria. Shattock [87] experimented on this question and found it was impossible to raise the virulence of lowly virulent diphtheria bacilli by cultivating them in a current of sewer air, even after two months.

In any consideration of this question Cobbett's observation "that in no case, as far as is known, has a virulent diphtheria bacillus been replaced by a non-virulent one before its final disappearance" from the throat, is worthy of note.

How many consecutive negative examinations are to be deemed necessary before infected persons can be released from isolation?

After deciding what types of bacilli are to be regarded as dangerous, the question as to the number of consecutive negative examinations that should be held necessary before release from isolation has to be considered.

The need for more than one negative examination has been very clearly established.

Hill [47] states that the Boston Board of Health, U.S.A., require two consecutive negative examinations of convalescents, and three for hospital patients, before they are declared free from infection.

At the South-Western's Fever Hospital, London [43], the patient is detained till the bacilli disappear as evidenced by three consecutive daily examinations.

Cobbett [18] requested the practitioners to submit swabs till three consecutive negative examinations were obtained. He found on more than one occasion that two consecutive negative examinations were followed by the discovery of the bacilli.

Out of 104 convalescent patients carefully examined at Colchester [41] on many occasions prior to discharge, one negative followed by the finding of diphtheria bacilli occurred in 11, two consecutive negatives in 10, three consecutive negatives in one, and four in one. Amongst 45 healthy infected contacts on eleven occasions diphtheria bacilli were again encountered after one negative, on three after two consecutive negatives, and once after four. In all 38 cases in which one or more negatives were followed by the finding of diphtheria bacilli. Many of these persons, both convalescents and contacts, retained their bacilli for long periods after one or more negatives had been obtained.

These misleading negatives may be due to the taking of swabs too soon after the application of some antiseptic, or to the bacilli lurking in the sinuses connected with the nasal cavities and finding their way thence into the pharynx.

Wolff [100] in 1895, examined the accessory sinuses of the nose in 22 fatal cases of diphtheria, and found diphtheria bacilli in 12, namely, once in the frontal sinus, six times in seven examinations in the sphenoidal, and twelve times out of fifteen examinations in the antrum.

Councilman, Mallory, and Pearce [26] found diphtheria bacilli in 21 (40%) out of 52 cases of inflammation of the antrum, and in 19 (51%) out of 38 examples of middle ear disease following diphtheria.

These figures show that two consecutive negatives, and in some cases even three, are not a complete safeguard. In practice, however, it is occasionally difficult to enforce isolation till three consecutive negatives have been obtained, and to insist on more would be impossible. Therefore, I think, whenever practicable, a minimum of three consecutive negative examinations should be enforced before convalescent cases, or, infected contacts, are freed from isolation.

The hearty cooperation of the medical practitioners, school authorities, and the majority of parents was obtained in the Cambridge [18-20] and Colchester [41] epidemics, when the principles of the measures which were being undertaken had been explained to them.

The experiences of various authors seem to indicate that in different epidemics the average length of time during which the bacilli persisted has varied greatly. According to some, antiseptic treatment of the throat and nose has had a very beneficial effect, others have not been able to detect much benefit. At Colchester many methods were adopted to shorten, if possible, by active treatment the period of detention. In some cases good results seemed to follow, but in others this was certainly not the case, and I came to the conclusion that although antiseptic treatment might be of benefit in limiting the power of the contact to infect others, yet the duration of the stay of the bacilli was not materially affected.

Park [70] seems to have had the same experience.

Bissel [10] considers that the average length of time for which diphtheria bacilli remain is 14 days.

The Massachusetts State Board of Health [66] found the average duration to be over 28 days, ranging from 7 to 84 days.

In Cobbett's [18] cases the bacilli seem to have persisted in the throats of infected persons (reckoning from the date of first examination to that of first of the 3 consecutive negatives) on the average during a period of 18 days. The longest period was 49 days, and the shortest 3.

In the spring of 1901 [20] he found virulent bacilli in the throat of a contact after 105 days.

At Colchester [41] amongst hospital patients, if a few exceptional cases be excluded, the mean duration of the period during which the diphtheria bacilli were found to persist was 28 days[1] from the date of notification, but in some exceptional cases they lingered up to 87 days. A few of the healthy children found to be harbouring diphtheria bacilli also retained them for long periods, in one case up to 94 days.

Welch [96] cites the work of Park in 1894. The latter examined 752 cases of diphtheria with reference to the lengths of time the diphtheria bacilli remain in the throat. In 325 cases they were only described for 3 days after the disappearance of the exudate; in 201 for 5 days; in 84 for 12 days; in 69 for 15 days; in 57 for 21 days;

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[1] Or 24·5 days after the disappearance of the membrane.

in 11 for 28 days; and in 5 for 35 days. The mean duration in these cases was therefore only about 8 days after the disappearance of the exudate.

In a later case the same author found diphtheria bacilli 49 days after the disappearance of the membrane.

Morse[68] in 25 cases found the average duration of the presence of the diphtheria bacilli after the disappearance of the membrane to be ten days.

No statement is made by the last two observers as to whether one or more negative examinations were required to prove the final disappearance of the organism.

Wesbrook[97] found the bacilli to be present in a boy for 135 days.

Woodhead[101] in his examination of the patients in the Metropolitan Asylums Board Hospitals encountered 79 cases which retained their bacilli for 100, and two for 200, days.

The varying periods during which the bacilli persist are well illustrated by the statistics on this point which have been given. The period is of course much prolonged if three consecutive, and not two, or one, negative examinations are considered necessary as proof of their disappearance. It is this uncertainty of the length of time for which bacilli may persist, and the consequent inability of the bacteriologist to state how long any patient or contact may have to be detained, which renders the enforcement of isolation difficult. The fact that the bacilli disappear, and after one or more negatives reappear, causes disappointment and irritation to the friends, and is a factor which makes the enforcement of isolation even more difficult than the actual length of time of their presence in the throat.

What class of persons should be examined and, if found to be infected, isolated?

The figures which have been already given as to the numbers of diphtheria bacilli found in "contacts" show that the percentage is highest amongst the closest contacts (*i.e.* members of an infected family), generally considerably lower amongst children in an infected school, and least in more distant contacts.

It consequently follows that in all cases the members of the family in which a clinical case has occurred ought to be examined, and, if the patient is a child attending school, at least the other members of his or her class. It also is most certainly advisable, whenever the circumstances render it at all possible, to follow up and examine other

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children who may have played with the patient or otherwise have come in contact with him.

In epidemic times all suspicious, and if possible all, cases of sore throat should be subjected to examination, especially if amongst scholars of infected schools.

Persons who were supposed to be suffering from tonsilitis have on several occasions been found to be infected with diphtheria. These are not only a danger to the community but are liable to suffer from the effects of toxaemia, owing to the omission of antitoxic treatment. Cases are cited later in which such persons have been the means of spreading the disease unknowingly.

Patients suffering from affections of the nose, such as membranous rhinitis[1], have frequently been demonstrated to be the carriers of infection.

Faucial diphtheria has only seldom, however, been observed to have been contracted from cases of membranous rhinitis, though instances are recorded by Cobbett[18], Dowson[30], Park[75], and Ravenel[82]. On the other hand a case of membranous rhinitis has not unfrequently been observed to give rise to another of the same kind. Examples have been cited by Abbott[1], Concetti[25], Dowson[30], and Ravenel[82]; and Lieven[60] "reported one case from which he obtained an organism that when introduced into the noses of other children by means of tampons caused a similar disease in them."

Ravenel[82] collected 41 cases of membranous rhinitis in which there was a record of bacteriological examination. In 33 of these diphtheria bacilli were found. In about 20% of cultures from cases of this disease tested for their pathogenic action on guinea-pigs, the virulence has been proved to be low, and moreover it has frequently been observed that the cultures rapidly die. The result of the examination of 52 cultures for virulence is given below.

	Virulent	Attenuated
Abbott[1]	2	1
Baginsky[5]	1	0
Cobbett[18]	1	0
Concetti[25]	2	0
Dowson[30]	2	0
Lack[57]	23	0
Park[75]	0	5
Ravenel[82]	5	3
Stamm[89]	3	0
Townsend[91]	4	0
	43	9

[1] See note on membranous rhinitis. Cobbett, *Journ. of Hygiene*, Vol. I. No. 2, p. 232. Also Hunt[50].

Symes[90A] has recently observed diphtheria bacilli in 20 (87 %) out of 23 cases of atrophic rhinitis; morphology in culture was principally relied on. He examined also a series of noses of healthy children and adults, but found in them no long diphtheria bacilli. A second control series of noses examined by him of cases of ozoena, congenital and acquired syphilis, rhinitis sicca, and lesions other than atrophic rhinitis, showed no diphtheria bacilli. Two out of the 17 long diphtheria bacilli found were tested for virulence, and both were found to be virulent. The author does not mention the reaction of the organisms in glucose media, and appears not to sharply distinguish between the diphtheria and Hofmann's bacillus, saying that among the normal noses in 58 % " a short diphtheria-like or pseudodiphtheria type of bacillus was present in the nose."

In a few instances diphtheria bacilli have been discovered in conjunctivitis (Jessop[51], Stephenson[90], Eyre[33] and others), and in lesions of the skin (Gordon Sharp[40], Park[76], Townsend[91], Wright[102], and Müller[69]): but in both these situations diphtheroid bacilli have frequently been encountered. In the conjunctiva the Xerosis bacillus, whose relationship with the diphtheria bacillus I do not propose to discuss, has been investigated by many observers (Berger[7], Eyre[33], Lawson[58]), and organisms morphologically resembling diphtheria bacilli have been isolated also from various lesions of the mucous membranes, e.g. catarrh of the cervix uteri, cancrum oris, urethritis, and pyorrhoea of the gums (paper by Fullerton and Bonney[35] and discussion on it). In consequence of these facts all organisms resembling diphtheria bacilli found in lesions of the skin and mucous membranes must be thoroughly investigated before their identity with the diphtheria bacillus can be established.

Also in times of epidemics it would seem most desirable to follow up any group of cases in which the evidence points to a common source of infection, as by the purchase of articles of food at certain shops, and the persons engaged in supplying or making these articles and their families should be examined.

In regard to the selection of infected persons for isolation some of the recommendations of the Massachusetts Committee[63] might be followed with advantage; these have already been cited. Briefly, they advise the isolation of infected children, and persons dealing with children; the exclusion from work of infected persons trading in articles of food; but not the isolation of bread-winners. They suggest

that these latter should be warned of the danger they are to the public and instructed in the use of antiseptic mouth-washes.

The question of notification of infected contacts is discussed and answered in the negative by Cobbett [20].

How are the diphtheria bacilli communicated by infected persons to others?

The majority of the authors who have been already quoted strongly emphasize the danger to the health of the community by healthy infected persons, and occasionally cite examples.

In epidemic times, though of necessity many instances must have come to their knowledge in which the probable channel of infection could be traced, considerable difficulty is often met with in confirming these cases and excluding all other possible sources of infection.

I have therefore thought that some of the instances detailed in recent literature might with advantage be quoted since one authentic example carries more weight than the mere quotation of abstract opinion.

Bisset [10] relates a case in which a child who had had a mild sore throat two months previously went to pay a visit. Diphtheria occurred in the household visited, and was transmitted by this child, as far as could be ascertained. He states that many such fully authenticated examples have occurred in the city of Buffalo.

Burnett [14] mentions an interesting case. In a school a boy was supposed to be suffering from a severe cold. There was a nasal discharge, which had been observed to be staining the pillow since the first day of term. On bacteriological examination diphtheria bacilli were found. No other case of catarrh in the school had these organisms. After the discovery every boy and master (with one exception) was given a prophylactic dose of antitoxin. The master who had not been given the dose contracted the disease, but no one else.

The bacilli lingered in this boy's throat for three months in spite of treatment. Finally after three consecutive negative examinations he was allowed to return to school when the term commenced; but the master was kept away, as after one negative the bacilli were again found. Soon after the beginning of term a fatal case of diphtheria occurred, when the boy's throat was again tested and diphtheria bacilli were found in it, and in the throats of three others.

Auden [4] attended a child suffering from diphtheria eight days after birth. Diphtheria bacilli were isolated. The mother said she had had a very severe sore throat one month before the child was born which had continued to cause difficulty in swallowing until one week before confinement. No external source of infection could be discovered.

Peck [80] gives a good instance of the transmission of the disease.

A boarding-school had 50 boarders and 50 day scholars. On October the 5th two of the boarders had sore throats. Swabs were taken and diphtheria bacilli discovered. The day school was dispersed, and one boarder (A. B.) was allowed to go home on the understanding that she was not to return till bacteriologically free. A third case occurred on October the 8th. All persons in the house were then examined with negative results except two. The three cases and two contacts were then isolated.

On Nov. 5th all the scholars reassembled and all were bacteriologically free. A. B. returned on Nov. 12th, but went home on Nov. 16th, and on Nov. 19th developed diphtheria. It was then ascertained that she had not been examined, but had been using an antiseptic spray. On Nov. 18th five boarders were suffering from slight ailments and three were found to have diphtheria bacilli; swabs from the rest of the school showed 28 children harbouring the bacilli.

The incubation period in A. B.'s case was 5 weeks.

White [99] states that a child in a tenement house suffered from diphtheria, and cultures revealed virulent diphtheria bacilli for three months. After one negative the child was released from isolation, but two days later cultures from the throat of this child and two others, who had been in contact with him, showed diphtheria bacilli, although the latter were never ill. Two other children coming to the house were exposed to the latter for two days and then returned home. In five days one of these developed diphtheria. Other sources of infection were excluded.

Cobbett [18] gives an example in which the distribution of infection was traced to a boy suffering from chronic membranous rhinitis, in the discharge from which were many virulent diphtheria bacilli. All the members of the family also had diphtheria bacilli. The boy had been attending school during three weeks in this condition. Six out of eight boys in his class suffered subsequently from the disease.

I [41] have given at length an account of a small outbreak in a school probably introduced by a child suffering from what was regarded as only a mild sore throat.

Park [76] traced a group of cases of diphtheria to a candy-store kept

by a family in which a case of diphtheria had occurred.   Children who bought candy at the shop acquired diphtheria, and other children, who came in contact with the healthy children of this family at school, also developed diphtheria.

Two children in a milkman's family were found to be suffering from diphtheria; the other members of the family were examined with negative results.   Three weeks later diphtheria began to make its appearance amongst the milkman's customers.   The two men employed in milking the cows were finally examined and found to be harbouring virulent bacilli.   (Denny [29].)

The dangers to public health attendant on the free communication of infected contacts with normal persons are well brought out by these instances.

The ways in which infected persons may communicate their bacilli to other persons are very numerous.

The kissing of babies and children as a means of spreading the disease has been insisted on by Hewlett and Murray [46].

In schools, however, probably sweets, pencils, pens, slates, &c. which pass from one child to another, and especially the habit children have of placing their fingers, and such articles as pencils, in their mouths may explain the rapid spread of infection in such institutions; and the absence of such habits in adults may to some extent account for their relative immunity from the disease.

Cobbett [18] traced the spread of the disease amongst certain children in a class to the hours during which slates were used.

In this connection Bond [11] remarks that he has ascertained that in certain schools each child does not have its own slate, and that as a method of cleaning their slates licking is common.

Cobbett also gives an excellent illustration of the dissemination of the disease by means of pencils.   A boy, the day before he was taken ill of diphtheria, spent the evening with some neighbours. Four of the latter were examined, and diphtheria bacilli found in two boys, but not in a baby and a girl.   On inquiry it was discovered that the original boy and the two others had played at parlour cricket, and each had taken it in turn to score with the same pencil which often, doubtless, found its way into their mouths.

The results of such contact in schools can only be prevented by the isolation of the infected, the systematic disinfection of the various articles in general use, the limitation of the use of certain articles to each child, and by bringing to the knowledge of the teachers the

possible ways in which the disease may be spread, as well as the importance of supervision and cleanliness. In some countries the use of slates in schools has been prohibited on hygienic grounds.

Can Infection be carried otherwise than by Infected Persons?

The answer to this is undoubtedly in the positive; but as compared to the method of dissemination by personal contact the means is probably rare.

Instances of contagion caused by *milk* are not uncommon—one has already been cited.

Bowhill[12] in connection with an outbreak of diphtheria at Cardiff attributed to infected milk, isolated from the suspected milk a diphtheria bacillus, whose virulence for guinea-pigs was proved by Nuttall.

Klein[55] found a typical, and pathogenic, diphtheria bacillus in one sample of milk out of 100 examined. Careful inquiries failed to show its origin, or that any persons had acquired the disease by drinking the milk. He[53] has also made the important observation that these organisms multiply rapidly in stored milk.

Eyre[31,32] also isolated the bacillus from a sample of milk, and subsequently made observations on 5 organisms derived from milk resembling the diphtheria bacillus in appearance, but not in pathogenicity.

Dean and Todd[28] found virulent diphtheria bacilli in certain ulcers on the teats and udders of cows and in their milk. One child which drank this milk developed diphtheria, and some other persons had sore throats, probably diphtherial. They proved by experiment, however, that the lesions on the teats were probably due to a separate infection on which the diphtheria bacilli were superadded.

Littlejohn[62] in his report on the health of the city of Edinburgh stated that it was free from diphtheria at the end of May 1900. On May 29th there was 1 case, during the next week 30, and the week following 40. The milk supply in this district was mainly from one dairy. The dairy-keeper and his family had sore throats in which diphtheria bacilli were found. The milk supply was stopped, and the epidemic ceased.

Howard[48] quotes several milk epidemics, and investigated one at Ashtabula, Ohio. He failed to find the bacilli in the milk or mouths of the dairymen, but ascertained that the son of one of the latter had lately been suffering from a very severe sore throat.

Klein [53] in 1889 inoculated cows subcutaneously with diphtheria cultures and found that they suffered amongst other lesions from eruptions on the udders and teats. From the latter lesions and the milk (drawn off) with precautions against infection from them, and from the seat of inoculation he was able to recover the bacilli. Subsequently he carried out other experiments of a similar nature.

Abbott [2] repeated these experiments, but was unable to confirm Klein's observations either as to the eruptions on the udder, or as to the appearance of the bacilli in the milk. Ritter [54] similarly failed to show that they passed into the milk.

Klein [54] replied to Abbott's criticisms of his experiments, and pointed out the causes for his failure.

Carstairs [16] recounts a case of a father and son who were cornet-players being attacked by diphtheria. The instrument was put away. Four years later a younger member of the family having found the cornet played it and developed diphtheria in a week. There had been no other case in the district for eight months previously.

Trevelyan [92] gives an instance in which diphtheria bacilli were cultivated from a handkerchief, eleven weeks after it had been used by a child suffering from diphtheria.

Vincenzi [93] examined the holy water in churches during an epidemic, and found, amongst other bacteria, diphtheria bacilli. He demonstrated their presence by culture and by virulence tests.

At Colchester, as a precautionary measure, the cups were removed from the public drinking-fountains, and a little later the water was cut off to prevent the children drinking from the spouts.

Weichardt [95], on the other hand, examined various objects, such as walls, linen, &c. about diphtheria cases by means of damp swabs. 300 samples were collected from 50 parts of the sick room, and 250 from other parts of the house (22 rooms in all). Diphtheria bacilli were found only three times, and in each case from objects which had been in direct contact with the child's mouth.

Welch [96] showed that in many examinations of hospitals diphtheria bacilli were not discovered except in situations which had been infected by direct contact with the patient, or his discharges, and that the bacilli were not present in the air.

The diphtheria bacillus has also been discovered by Cobbett [22] in the *horse*. Its cultural peculiarities and virulence were fully tested.

The daughter of the owner was suffering from diphtheria and the condition was only brought to notice on this account.

Bacilli morphologically identical with the diphtheria bacillus have been described in chickens with a contagious disease called "roup" by Gordon Sharp [39] and also by Gallez [36]. The former points out some connections between the epidemic in chickens and the disease in man, but he thinks that the bacilli in fowls are less virulent than in man.

It should be pointed out however that diphtheria-like organisms have also been discovered in pigeons, both in normal individuals and those suffering from "pigeon canker." They produce acid in glucose broth and stain by Neisser's method, but are non-pathogenic for guinea-pigs (Macfadyen and Hewlett [64]). The disease known as avian diphtheria in France, which appears in very fatal epidemics in fowls, is however due to a completely different organism (Guérin [42]).

Klein [53] experimented on *cats*, and found that they succumbed to inoculations of diphtheria bacilli. Some of these animals suffered from respiratory troubles, others from paralysis and weakness, but all, at autopsy, showed marked changes in the kidneys, which were enlarged and had extensive areas of fatty degeneration in the cortex. He also found that certain cats accidentally fed with milk from the inoculated cows acquired the disease and transmitted it to others. He further fed cats experimentally on milk containing diphtheria bacilli, and found that they died of a similar disease, but in none of these cases did he apparently cultivate the organism from those animals. Abbott and Welch [3] by tracheal inoculations showed that kittens developed a pseudo-membrane in the larynx, and died suffering from respiratory trouble and great weakness.

Klein [53] cites an interesting case, reported by Dr Bruce Low, of the spread of diphtheria by a cat. "A little boy had a fatal attack of diphtheria. On the first day he vomited, and the cat licked up the vomit on the floor. In a few days (and after the death of the boy) the cat was noticed to be ill, and her sufferings became so severe and similar to those of the dead boy that her owner destroyed her. During the early part of its illness this cat was let out into the back yard; a few days later the cat of a neighbour who lived a few doors off was noticed to be ill. This cat had also been in the back yard at night. This second cat recovered, being carefully nursed by four little girls, all of whom developed diphtheria. There was no other known source of infection to which these girls had been exposed except the cat." He also enumerates several other cases of a similar description.

Diphtheria-like bacilli have also been isolated by Dowson [30] from cats suffering from illness during an epidemic of diphtheria at Bristol. These showed the kidney lesions described by Klein.

Although in the majority of instances the disease would not be likely to spread by infected animals yet the possibility of their conveying the bacilli, and of causing isolated cases, must be borne in mind.

The question as to how certain persons can harbour virulent diphtheria bacilli without being ill has been answered by the researches of Wassermann [94], Orlowski [73], and others. The former found considerable quantities of antitoxin (enough in 1 c.c. of serum to protect guinea-pigs completely against 10 fatal doses) in the blood of 50 % of children and 83 % of adults in 17 and 28 examinations respectively; and the latter found that 5 out of 10 children in a hospital had antitoxin in their blood. None of these persons as far as could be ascertained had suffered from diphtheria.

Fischl and Wunscheim [34] also found more or less antitoxic substance in the placental blood of 68 out of 82 infants (83 %); and Cobbett [23] found this substance in the blood of 8 out of 11 horses.

It is probably such immune persons who carry the bacilli and give rise under favourable conditions to epidemics.

The enumeration of the foregoing facts and the deductions based on them render it most desirable that further studies should be made into the ways in which the disease spreads, and the occurrence of diphtheria bacilli in healthy persons who have not been exposed to infection. It is for this reason that I have ventured to give the results of certain investigations on the occurrence of Hofmann's bacillus in the healthy throats of contacts and others, and the bacteriology of the mouths of normal persons (non-contacts).

Methods.

Swabs were prepared, constructed of cotton-wool and wrapped round a stout wire. These were placed inside stout glass test-tubes and sterilized.

In obtaining a culture for examination the throat, or nose, of the person was wiped with the swab, which was then returned to its case. As soon as possible the infected swab was rubbed over the surface of a serum tube[1]. The culture so obtained was grown at 37 % for 24 hours or less.

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[1] See footnote on p. 229.

At the time of examination samples from dissimilar colonies were streaked on coverslips by means of a sterilized platinum needle, stained by Löffler's methylene-blue (diluted 1 : 5), mounted in the stain and examined under a $\frac{1}{12}$ oil immersion lens (see Cobbett and Phillips [24]). Unless the growth was very scanty, or the culture was very thickly studded with colonies, organisms from more than one colony were never placed on the same portion of the coverslip. By this means the appearance of the various colonies and the morphology of the bacilli derived from them could be studied, and the difficulties in distinguishing the organisms in a general smear avoided.

In all cases in which suspicious bacilli occurred ·5 % acetic acid was run under the coverslip in the way suggested by Cobbett [21], or a separate preparation from a similar colony was stained by Neisser's method. In many cases the two methods were compared.

By these methods of staining it was found that in almost every case diphtheria bacilli were distinguishable by the presence of darkly stained polar bodies; whereas Hofmann's bacilli had none. It is true that certain examples of the diphtheria bacillus showed no polar bodies[1] by the acetic acid or Neisser methods, and that inconspicuous and scarce polar bodies were sometimes seen in Hofmann's bacillus. Also certain bacilli[2] and cocci were encountered which showed polar bodies by both these methods. These facts do not, however, materially detract from the value of the method, which is a considerable aid to diagnosis, so long as it is not trusted to alone.

Cobbett [41] had previously come to the same conclusion. Beaton, Caiger, and Pakes [6] have experimented with Neisser's stain and are convinced of the utility of the method, and Cammidge [15] agrees with them.

Hewlett [44] considers that " with precaution to exclude fallacies, and using fresh membrane, Neisser's method will often afford a means of rapid diagnosis."

Besides the tests which have been already given, a large number of pure cultures of both diphtheria and Hofmann's bacilli were grown in glucose broth. The former without exception formed acid, the latter did not.

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[1] The absence of polar bodies is not an indication of want of virulence—one example of the diphtheria bacillus which neither in the original, nor subsequent, cultures showed them was virulent.

[2] I hope to call attention later to the occurrence of polar bodies in certain other organisms resembling diphtheria bacilli when treated by these methods.

A few examples of each were tested for virulence. The results were exactly in accord with those of Cobbett, namely, a small portion of diphtheria bacilli were found to be non-pathogenic, but the majority were pathogenic in doses of ·1 c.c. Hofmann's bacillus was not found to have any pathogenic properties, whether the typical, or pseudo-diphtheria, forms were tested.

The pseudo-diphtheria form of Hofmann's bacillus is one of the few organisms met with in Cobbett's experience, or my own, which is often likely to be mistaken for the diphtheria bacillus. Even when these are present I completely agree with Cobbett when he says, "it is possible to train the eye to distinguish with a sufficient degree of precision the diphtheria bacillus from the bacillus of Hofmann" (see p. 229).

The want of accord in the positive and negative diagnoses of cultures given by the various bacteriologists who made observations for the Massachusetts Committee[65] as compared with the Committee's standard seems to mostly depend on whether, or not, they considered Hofmann's bacillus in its various forms to be a causal agent in diphtheria.

Most of the other observers who have been quoted give statistics detailing the number of times they have found this organism and appear to attach some importance to its presence. Pakes[74], for example, thinks it capable of giving rise to a specific sore throat.

Cobbett on the other hand, as has already been seen, comes to the conclusion that it is not in any way connected with disease in man. He says[20] "my experience of the outbreak of diphtheria at Cambridge (1900) gave no reason for thinking that the pseudo-diphtheria bacillus (Hofmann) is other than perfectly innocuous to man." And his observations in the Spring epidemic of 1902 confirmed these views. I have found this bacillus in a large number of cases, and agree with him both in the above opinion and in his statement that it is a common inhabitant of the mouths of the poorer classes.

Its non-occurrence to a great extent in the mouths of patients suffering from diphtheria is, I believe, to be explained by the fact that during epidemics, once the diphtheria bacillus has been found, no further search is made, but when the latter organism is disappearing a prolonged search is often necessary, and in the course of it the bacillus of Hofmann is encountered and recorded. Hence the view is likely to be entertained that it is an attenuated form of the diphtheria bacillus. Cultures of this organism from persons in every stage of convalescence

from diphtheria have been isolated by Cobbett[19] and all were found to be equally harmless.

Regarding the bacillus of Hofmann as a common innocuous inhabitant of the mouth I think its prevalence can be made to illustrate the manner in which diphtheria bacilli are carried from mouth to mouth.

During the outbreak at Colchester I examined 576 cultures from non-infected contacts of all classes and found the bacillus of Hofmann on 316 occasions (54·8 %). The great majority of the persons examined belonged to the poorer classes, and the bacillus was of very frequent occurrence in their throats, whereas in the more well-to-do persons the percentage of cases in which it occurred was much lower.

In schools attended by the children of the poor where many articles are shared in common, and want of strict attention to cleanliness is frequently observed, 64·5 % harboured this bacillus (411 examinations).

The following table gives the percentage occurrence of various common organisms in the mouths of non-infected contacts amongst school-children and others.

Class of persons examined		No. of cultures	Percentage of				No. of healthy infected persons not included in Nos. given	
			Hofmann	Staphylococci	Streptococci	Oval bacilli		
Scholars : School	I	6	66·6	16	0	0	0	These schools were attended by the children of the poor
,,	II	50	64	56	2	2	8	411 cultures examined. Hofmann's bacillus found in 64·5 % of examinations
,,	III	49	63·3	49	0	2	8	
,,	IV	149	63	67	2	30	6	
,,	V	59	62·7	49	4	2	5	
,,	VI	16	62·5	44	6	13	1	
,,	VII	15	60	60	0	7	5	
,,	VIII	37	57	66	0	9	5	
,,	IX	30	56·6	53	0	0	0	
,,	X	9	33·3	67	0	11	1	Better class schools. 19 examinations. Hofmann bacillus found in 31·5 %
,,	XI	10	30·0	90	0	0	0	
Persons above school age		40	50	43	8	5	3	
Persons above 20 years of age		13	30·7	61	8	8	2	Included in above list. Mostly parents of scholars in schools I—IX
Below school age		14	42·8	79	0	0	0	
Well-to-do persons		79	22·7	68	8	12	23	

The above table shows how very commonly the bacillus of Hofmann is found in the throats of non-infected contacts. Among the scholars in

the poorer schools its range is from 66·6 to 56·6 %. Here the opportunities for spreading are very great by the various methods that have already been described. Amongst older and younger persons of the same class who are liable to acquire the organisms in similar ways, but not to the same extent, its range is from 50—42·8 %. Thirteen persons, the parents of these children, harboured it only in 30 % of cases.

In the rather better class schools it is present in much smaller numbers, namely, in 33 % and 30 % of individuals (only 19 persons however were examined), and amongst the well-to-do class only 22·7 % were found to harbour this organism in their mouths.

The latter, moreover, were in nearly all cases closely related to diphtheria patients, and consequently a large number of infected contacts were found amongst them.

In order to ascertain to what extent Hofmann's bacillus was present amongst the members of poorer families, where the children often attend different schools, the following table was constructed.

Families not infected with Hofmann's bacillus		Families in which Hofmann's bacillus was found in at least one member		Families with every member infected with Hofmann's bacillus		
No. of persons per family	No. harbouring Hofmann	No. of persons per family	No. harbouring Hofmann	No. of persons per family	No. harbouring Hofmann	
6	0	5	4	8	8	
5	0	5	1	4	4	
4	0	4	3	4	4	
3	0	4	3	3	3	
3	0	4	3	3	3	
3	0	4	2	3	3	
3	0	4	2	3	3	
3	0	4	1	3	3	
2	0	3	2	3	3	
2	0	3	2	2	2	Total percentage of persons harbouring Hofmann's bacillus in families infected with this organism 73·6 %. 66 families examined.
2	0	3	2	2	2	
2	0	3	1	2	2	
2	0	3	1	2	2	
2	0	3	1	2	2	
2	0	2	1	2	2	
2	0	2	1	2	2	
2	0	2	1	2	2	
2	0	2	1	2	2	
2	0	2	1	2	2	
2	0	2	1			
2	0	2	1			
		2	1			
		2	1			
58	0 (0 %)	72	38 (52·8 %)	56	56 (100 %)	

The results are somewhat remarkable, and show that when the organism has once been introduced into the family many of the younger members acquire it. Dr Cobbett very kindly placed at my disposal his records of the results of his examinations. I find that he has noted the organisms discovered in 1495 examinations in which diphtheria bacilli were not found. He recorded the presence of Hofmann's bacillus in 35·9 % of these observations.

His records[1] resemble mine to a striking degree as far as the distribution of this bacillus in school-children and well-to-do persons is concerned. Its range in three poor schools was from 78·9—63·3 %, whereas amongst the undergraduates of Sidney Sussex College, Cambridge, it only occurred in 21·9 % of individuals.

To the following table giving Cobbett's results I have added those of the recent examinations of two schools by myself.

| Class of persons examined | No. of cultures | Percentage | | | | Healthy contacts |
		Hofmann	Staphylo-cocci	Strepto-cocci	Oval	
Non-infected contacts	1495	**35·9**	65	24	5	?
Scholars :						
Poorer school I	19	**78·9**	84	21	15	1
,, ,, II	49	**75·5**	38	2	0	0
,, ,, III	120	**63·3**	62	13	2	8
*Better class school IV†	29	**0**	66	10	17	0
* ,, ,, V	49	**12·2**	71	25	39	1
Undergraduates of Sidney Sussex College	41	**21·9**	78	20	10	3

    * Examined by me, 1902.   † School situated in the country.

In a total of 2198 examinations of non-infected contacts by Cobbett and myself the bacillus of Hofmann was found to be present on at least 870 occasions (nearly 40 %).

It was found comparatively infrequently in the mouths of well-to-do persons (13·3 % of 217 examinations), and very commonly amongst the poorer children (65·7 % of 599 examinations).

The vast majority of these persons were in perfect health, but a few had sore throats at the time of examination. So far as I can ascertain none suffered from diphtheria within several months.

[1] At the time of the various examinations made by Dr Cobbett and myself the organisms observed were recorded, but no idea of classifying them was at the time entertained. Except in the case of Hofmann's bacillus the percentages given are probably too low, since every class of organism present was not always recorded.

I think the following deductions may be drawn from these statistics.

(1)   That the bacillus of Hofmann (as previously defined) is perfectly innocuous to man.

(2)   That it is a common inhabitant of the mouths of the poorer classes, especially children.

(3)   That it is relatively uncommon amongst well-to-do persons, and even amongst the children of this class.

(4)   That it probably spreads from one child to another by the means, that have been indicated as the probable ones, by which diphtheria bacilli are transferred from one individual to another.

(5)   That in the absence of diphtheria bacilli morphologically resembling Hofmann's bacillus, described by Wesbrook in an outbreak at Owatonna, but which have never been met with by Cobbett or myself, no importance whatever should be attached to the presence of Hofmann's bacillus.

Organisms found in the mouths of healthy persons who have not been exposed to the disease.

In view of the satisfactory results which have followed in those outbreaks in which strict isolation of convalescent patients and healthy infected contacts has been enforced, and the theoretical and practical importance of ascertaining whether virulent diphtheria bacilli are to be found in the mouths and noses of healthy persons, who have had no opportunity of acquiring these bacilli by contact with the disease, I have tabulated the results of examination of some of these persons.

It has been pointed out earlier that when investigations of this kind have been carefully conducted, and measures taken to test the virulence of organisms, apparently identical with the diphtheria bacillus, which have been discovered, and also to inquire into the history of persons from whom virulent diphtheria bacilli have been isolated, the proportion of cases in which inquiry did not lead to the discovery of recent contact was very small, namely, 3 ($\cdot 26\,^{0}/_{0}$) in 1149 persons examined.

Remembering the great difficulty often met with in prosecuting inquiry amongst the class of persons from whom hospital cases are drawn, and amongst whom these investigations were principally conducted, these figures are very striking, and in the absence of further

evidence undoubtedly point to the conclusion that virulent diphtheria bacilli seldom, if ever, exist in the mouths of the normal population.

In the following tables are given the results of the examination of 362 persons, several of whom were examined more than once. Some were workers in the pathological laboratory; some undergraduates in the University; some patients in Addenbrooke's Hospital; but the larger number were persons of the poorer classes, suffering from sore throats, and their friends.

Swabs were sent up for examination from a few of the latter persons by medical practitioners in attendance, but in the majority of cases an examination was only made because I desired to ascertain what organisms were present. The patient was usually able to procure swabs from friends also. In every instance here given inquiries were made as to any possibility of contact with recent diphtheria cases, or healthy infected contacts, and only cases, in which no probability of such an occurrence could be ascertained, are inserted here. In the few cases in which the history pointed to a possibility of contact the results have not been recorded, though all were negative.

All these persons may then be regarded from our point of view as normal individuals.

In this series a considerable proportion of the persons examined were above the age at which diphtheria is most common. This is to be regretted; but I hope at a later date to be able to record the results of examinations of healthy children.

In no single instance amongst these 362 persons was a virulent diphtheria bacillus met with, nor any organism that could not be distinguished morphologically and by staining methods, with the exception of one non-virulent diphtheria bacillus.

The few organisms which slightly resembled diphtheria bacilli were isolated in subculture, and tested for their power of producing acid in glucose broth, and for virulence.

In view of certain observations which have been quoted it is most desirable that as far as possible in all investigations on healthy throats the virulence of all organisms morphologically and culturally resembling diphtheria bacilli should be tested; and that when virulent bacilli are found, the effects of simultaneous injection of antitoxin should be ascertained[1].

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[1] I have lately found an organism resembling the diphtheria bacillus in its morphology. It forms acid in glucose broth and gives a positive Neisser reaction, and kills guinea-pigs in 24 hours. On post-mortem examination however the organisms were cultivated from

Since the diphtheria bacillus acts both on man and guinea-pigs by means of its toxine, and non-virulent bacilli produce no toxine in culture, without definite proof to the contrary it may be inferred that the non-virulent diphtheria bacillus is harmless to man. At present this appears to be the opinion of most authorities.

Table showing the organisms found in the mouths of healthy non-contacts.

	Persons examined	Hofmann's bacillus	Staphylo-cocci	Streptococci	Oval bacilli	Diphtheria bacilli
Members of the University	48	**4·1** %	77 %	16·6 %	27·0 %	0
Patients in Addenbrooke's Hospital	98	**12·2**	63·3	8·1	30·5	1
Workers in the Patho-logical Laboratory	18	**22·2**	44·4	55·5	66·6	0
Other persons	198	**24·7**	77·7	13·1	22·2	0
Total	362	**18·5**	72·9	14·8	27·3	·27

As has already been stated most of these persons were adults, who are apparently not so liable as children to acquire Hofmann's bacillus, since daily congregations of individuals as in schools do not occur, and the habit of placing various articles in their mouths is not so common as amongst children. The table, however, shows that nearly a quarter of persons of the poorer class, placed under the last heading, harboured these organisms in their mouths, while but 4 % of the members of the University did so. It is also seen that the hospital patients, who belonged to the same class as the former, harbour them only to the extent of 12 %. This seems to be due to the fact that the majority of the latter were country people, and consequently less likely than persons of their own class in towns to acquire bacilli by contact.

The one example of the diphtheria bacillus, and that a non-virulent one, was met with amongst the hospital patients. No history of contact could be obtained.

On combining these observations with those of the other workers mentioned (page 219) who have made inquiries as to the possibility of recent contact and worked out the virulence of the organisms they observed, it is found that amongst 1511 persons virulent diphtheria bacilli were only isolated on two occasions, and non-virulent on thirty.

the heart's blood and organs, and numerous microscopic haemorrhages crowded with bacilli were found on section. In this respect, and in the appearance of its colonies, it differs from the diphtheria bacillus. This is apparently the organism described by Davis[27] in 1898.

I have also found in the mouth diphtheroid organisms resembling those described by Foullerton and Bonney[35].

Also in one case bacilli morphologically identical with diphtheria bacilli, but too few to be subcultured, were seen. Including the doubtful one the percentage of virulent diphtheria bacilli met with amongst these persons is ·19, and of the non-virulent 1·98.

Summary.

1. Diphtheria bacilli have been found in a considerable proportion of persons who have come into contact with cases of diphtheria or with other infected persons.

2. Such persons have been shown to be a grave danger to public health, especially when frequenting schools or institutions, and to constitute the usual channel by which the disease is spread.

3. Very satisfactory results have followed on the isolation of convalescents from the disease and of infected "contacts," where two or more consecutive negative examinations have been required before release.

4. Carefully conducted investigations amongst healthy persons, who have not at a recent date been in contact with diphtheria cases or infected "contacts," have shown that virulent diphtheria bacilli are very seldom (3 examples amongst 1511 persons) present in the mouths of the normal population. This fact renders the discovery and isolation of infected persons a practicable possibility and offers a fair prospect of discovering and isolating the majority of them in any outbreak.

5. Diphtheria bacilli are usually distinguishable on morphological and cultural grounds, but whenever possible it is desirable that their virulence should be tested.

6. The bacillus of Hofmann is innocuous to man, and is a very common organism in the mouths of the poorer classes. The distribution of this bacillus points to the conclusion that it is carried from mouth to mouth in the same way as the diphtheria bacillus, and therefore its widespread prevalence in schools attended by poorer children is significant, as showing how widely spread and uncontrollable an outbreak of diphtheria may become unless measures are early taken to deal with infected contacts.

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THE BIOLOGICAL OR PRECIPITIN TEST FOR BLOOD CONSIDERED MAINLY FROM ITS MEDICO-LEGAL ASPECT[1].

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CONTENTS.

A POINT of great importance in medico-legal practice is the differentiation of human from other bloods. In dealing with fresh stains no difficulty has been found in distinguishing mammalian from avian and reptilian bloods. The more difficult operation of differen-

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[1] The work on that portion of this paper which deals with the detection of blood dried on various fabrics and leathers, and the effects of age, soil, and certain chemical reagents (qualitatively) was carried out by Mr Sanger, partly in conjunction with myself, as the subject of his Thesis for the Degree of M.D. Subsequently I repeated our previous work and added all the quantitative determinations. We are indebted to Dr Nuttall for suggesting the main lines of this investigation and for the aid he has given us in our work.

G. S. G.-S.

tiating between mammalian bloods, for example between human and pig's blood, had up till the introduction of the biological, or preferably precipitin, test, except with the freshest material, baffled the ingenuity of the expert in legal medicine. In 1901 Nuttall (1. vi. '01) was amongst the first to draw attention to the possible value of this test for blood and serum in forensic practice.

Uhlenhuth, Ziemke, Biondi, and others on the Continent have worked extensively on the medico-legal side of the question, and the test has now been used in the courts of Germany, Italy, Spain, Norway, Roumania, Egypt, and the United States.

In this country, however, with the exception of Nuttall and Grünbaum, none have yet worked systematically on the subject in any of its aspects, and in forensic practice it has been completely neglected.

Since their discovery the study of precipitins has attracted many investigators, and in consequence an immense number of observations have been made on the subject. The greater part of this work has been undertaken to elucidate points of scientific interest, and has been carried out with fresh material under the most favourable conditions. A study of the literature, however, reveals the fact that the various authorities are not all in agreement on some points of practical importance, as for example the effects of varying strengths of salt-solution, and of acidity and alkalinity. In a paper to be published shortly Nuttall has summarised the literature on the " precipitins " and other allied bodies, and recorded the results of very extensive observations on " the blood-relationship of animals."

Uhlenhuth, Wassermann, Ziemke, Biondi, and others have directed their attention to the application of the precipitin test in forensic medicine, and have studied the effects of many of the conditions subject to which the test would have to be applied in practice. Their researches have conclusively established the value of this method of determining the identity of blood-stains under a number of more or less adverse circumstances[1].

Up to the present no work has been done in measuring accurately the amount of " precipitum " formed, and thereby estimating the effects of various reagents and conditions on blood to be tested, and on antisera.

In this paper we have endeavoured to determine by the quantitative method introduced by Nuttall (5. iv. '02) the effects of age, heat,

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[1] Working with 1 in 100 dilutions of pure serum Nuttall (vi. 1901) has shown that it is possible to distinguish the several bloods in a mixture.

putrefaction, filtration, and contact with soils, and chemical reagents, both on normal blood sera, and also on antisera. We have also applied the test to blood dried on various materials on which it might be met with in forensic practice, and have in most cases been able to devise methods to eliminate the various sources of error, due to the nature of the substances on which the blood has been deposited.

These experiments have led us to the conclusion that with sufficient materials, and due precaution to exclude the various sources of error, there are but few conditions met with in forensic practice under which human could not be readily differentiated from other bloods. By this, however, we do not mean to imply that a considerable acquaintance with the action of precipitating antisera on blood solutions is not necessary in the successful application of this test.

A summary of the most striking of the cases in which this test has been applied in medico-legal inquiries will be given later.

Methods of producing Antisera.

It has been found that the result of the injection of the serum of almost any animal into a rabbit is to produce in the blood of that rabbit a body, which brings about the formation of a precipitate, when the latter's serum is mixed with the diluted serum of the former. This precipitate is technically spoken of as a "precipitum," and the serum of the treated animal as an antiserum to the species of animal from which the injected blood was derived.

The technique of the preparation of antiserum has been fully described by many workers, and is exhaustively considered by Nuttall in his paper to be published shortly.

In these experiments the anti-human serum, and some of the others, were prepared by intravenous injection, but much smaller quantities than those usually employed were found to suffice[1]. For example 18 c.c. of human serum injected in doses of 5, 5, 5 and 3 c.c. at intervals of 2, 3 and 4 days produced a powerful anti-human serum. The animal was bled 14 days after the last injection. Continental workers have used quantities ranging to hundreds of c.cs., and have frequently found that their animals stood the operation badly, whereas the above animal

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[1] Powerful anti-ox and anti-sheep sera were made by injections of 9·2 and 12 c.c. in doses of 1, 2, 1, 3·2, and 1·5, 2, 2, 3·5, and 3 c.c. respectively. The intervals between the injections were 4, 4, 2 and 5 days in each case, and the animals were bled 7 and 10 days after the last injections.

and most of the others we have treated, continued to gain weight, and appeared to be healthy. Some of our control antisera were prepared by the intra-peritoneal method and quantities ranging from 30 to 60 c.c. were used. The animals stood the treatment very well.

Methods of preserving Sera and Antisera.

Many methods have been adopted by various observers for storing sera and antisera. The plan which we have always adopted in the case of antisera is as follows :

The blood is collected with all precautions against contamination in a large sterile Petri dish, which is tilted after the formation of a firm clot. The serum, which is expressed from the clot, is drawn up into small tubes about ·5 cms. in diameter and 10 cms. in length with the ends drawn out into fine capillaries. When full the ends are sealed and the tubes stored in an upright position. By this method a few drops of antiserum can, if necessary, be used at a time, and the tube again sealed without contamination. We have not noticed that antisera so sealed, and preserved at room temperature in the light, lose their properties any sooner than those kept in the ice-chest in the dark.

Chloroform, Trikresol, and Carbolic Acid have been used as preservative agents by some observers.

Fluid sera when obtained in a sterile condition were usually sealed in glass bulbs without the addition of preservatives, and when received in a putrid condition were similarly stored after filtration through porcelain.

Some fluid sera, especially those which were likely to have been contaminated, were stored in bottles with closely fitting stoppers, and putrefaction checked by the addition of a few drops of chloroform.

Blood dried on filter-paper and various fabrics has usually been kept in the dark at room temperature.

Methods of testing.

Qualitative.

When fluid serum could be obtained in sufficient quantities dilutions of 1 in 21 in ·6 % saline solutions were generally used for testing. For testing samples of blood dried on various materials extracts were made by placing blood-stained pieces of these for some hours in small

quantities (1—2 c.c.) of distilled water, and afterwards adding equal volumes of $1\cdot2\,^0/_0$ salt solution. These extracts were generally found to be slightly tinted and clear, and to foam well on shaking.

In testing such solutions about $\cdot5$ c.c. were placed in small test-tubes of about 1 c.c. capacity contained in racks provided with a black background, and one to two drops of antiserum were run into each tube. Owing to its greater specific gravity the antiserum immediately flowed to the bottom of the tube. When the antiserum corresponded to the blood which was being tested a white cloud appeared at the junction of the fluids within a few minutes, and gradually developed into a dense cloud. After a few hours a well-marked precipitum was found at the bottom of the tube. In the following experiments controls were used in all cases. For example when tests were being made from suspected human blood at least two tubes of the extracted blood were prepared, to one of which was added anti-human serum, and to the other anti-ox or other antiserum. Frequently three or more controls were employed, normal rabbit, anti-horse, anti-ox, anti-sheep, anti-dog, anti-hedgehog, and anti-turtle sera being used for this purpose. If human blood was alone present the cloudings and precipitum appeared only in the tube to which anti-human serum had been added. Occasionally very faint cloudings and traces of deposit occurred in other tubes, used as controls, to which mammalian antisera had been added, indicating the "mammalian reaction." Nuttall (21. xi. '01; 20. i. '02) was the first to call attention to this mammalian reaction, and to show that the precipitating antisera are not strictly specific, but produce reactions with the bloods of nearly allied species. He pointed out that the more powerful the antiserum the more likely it is to include in its action distantly related animals.

Except in the case of closely allied species the cloudings resulting from this cause are slight, and from the point of view of forensic practice in this country may be neglected, though in lands where old-world monkeys are common it might be necessary to be provided with antisera for the prevalent species.

In fact it may be said that except under abnormal conditions antisera produce marked cloudings with their homologous sera only. The causes of the abnormal conditions just mentioned, when all antisera react more or less markedly in the same way, will be dealt with later.

This may be called the qualitative method.

Quantitative method.

In medico-legal practice fluid material would but rarely have to be tested, and consequently the qualitative method just described would have to be relied on.

For estimating the effects of age, putrefaction, and chemical reagents on fluid sera and antisera, the quantitative method devised by Nuttall (5. vi. '02) has been employed. The method has been fully described by him, but a short description is necessary here.

By means of an accurately graduated pipette ·5 c.c. of a 1 in 21 dilution of serum in normal salt solution is placed in a small, clean, dry test-tube, and later ·1 c.c. of antiserum is run in. The fluids are then thoroughly mixed by inverting the test-tube with the mouth closed by a carefully dried finger. This mixture is allowed to stand for 24 hours, by which time the precipitum has settled to the bottom of the tube. Should any have adhered to the sides it can be removed by gentle rotation or tapping. The supernatant fluid is then pipetted off, and the precipitum drawn up into a capillary tube (Plate XI. fig. 1[7—9]). One end of this is sealed, and it is allowed to stand for 72 hours for the precipitum to again settle. By means of the instrument devised by Nuttall the volume of the capillary tube occupied by precipitum can now be estimated.

When using this method controls treated in the same way with other antisera were employed on several occasions. In other experiments only qualitative controls were made use of.

It is obvious that in these manipulations there are many possible sources of error unless the greatest care is exercised; with accurate dilutions and measurements of the quantities of fluid added together, most of these can, however, be excluded. If the diameter of the capillaries used is fairly constant, the only marked source of error which remains is the condition of the precipitum. Some precipita are flocculent and occupy much space, others pack into a fairly solid mass. It has been noticed, however, that either flocculent or compactly formed precipita are produced by the serum under examination, and that unless the conditions of the experiment are markedly changed the same serum always produces the same kind of precipitum.

In order to determine the range of experimental error in carefully made observations series of experiments were carried out, the details of two of which are given below. The results in all cases, both here and elsewhere, are expressed in cubic centimetres.

*Results of measurements of precipitatum from four samples of a 1 in 21
dilution of human blood and 8 samples of a similar dilution of ox
serum, to which ·1 c.c. of their homologous antisera had been added.*

Human serum		Ox blood		
·0281 c.c.		·0215 c.c.	·0233 c.c.	
·0281 „	Mean ·0293 c.c.	·0225 „	·0233 „	Mean ·0233 c.c.
·0300 „		·0233 „	·0233 „	
·0309 „		·0233 „	·0262 „	

The fluctuations above and below the means in the human series
are ·0012 c.c. or 4 %, and in the ox series ·0029 c.c. and ·0018 c.c.
respectively or 12 % and 7 %.

In order to arrive at the most trustworthy figures possible in most
experiments two observations were made under identical conditions in
the hope that by this means the experimental error might be reduced
to a minimum.

In measurements of this kind, made with every care, probably a
margin of 10 % must be allowed for experimental error.

Throughout these experiments the aim has been to indicate by
measurements the effects of varying conditions on the formation of the
precipitum as compared with controls. It must be stated, however,
that with every precaution the measurements of the same set of
materials on different days are not identical, although the proportions
which the various members of the set bear to each other remain fairly
constant. Hence though improvements in the technique of measurement
may result in more accurate and constant figures, yet it is improbable
that the general results will be materially altered.

Sources of error connected with properly preserved (1) *Antisera and*
(2) Sera to be tested.

(1) *Antisera.*

Opalescent antisera. Of the continental workers, Uhlenhuth has
published warnings against the use of opalescent antisera, which give
cloudings with nearly all bloods, as has also been found by Nuttall.
Uhlenhuth (11—18, IX. '02, p. 680) considered that they might be due
to the animals being bled too soon after a meal. In this laboratory
animals have been bled at all times, and very few opalescent antisera
have been met with. We have noticed that in some cases the animals
from which these opalescent antisera have been obtained have suffered
from disease of the liver caused by *Cysticerci*. The very large injections
practised by some foreign observers may account for some opalescent
sera.

Filtration through porcelain, as Uhlenhuth (11—18. ix. '02, p. 680) and Rostoski (1902 b., p. 29) have shown, produces no effect.

Whatever the cause of this opalescence may be the sera are certainly untrustworthy.

Very powerful antisera may lead to false conclusions from producing comparatively large reactions in allied, or even distantly related bloods. A control tube containing homologous blood in a dilution approximately equal to that of the blood under examination would sufficiently guard against any mistake from this source.

Very weak antisera could only lead to the error of making a negative diagnosis when its homologous blood was in reality present.

Certain antisera which have been stored even in sealed bulbs kept in the dark in the ice-chest develop the property of causing cloudings in all serum dilutions to which they are added. Under these conditions they become utterly worthless.

(2) *Sera to be tested.*

Nuttall found that two specimens of monkey blood of *Cynocephalus* and *Macacus* caused with human antiserum precipita nearly equal to those of human blood. On enquiry as to the cause of death it was found that the former had died of intussusception, and the latter of dysentery, both diseases tending to produce concentration of the serum during life.

Measurements with anti-human serum by Nuttall and Strangeways[1].

Human	serum	...	100 %	
Macacus	,,	...	90 ,,	died of dysentery
Ourang	,,	...	80 ,,	
Cynocephalus	,,	...	70 ,,	died of intussusception
Mandrill	,,	...	50 ,,	(healthy)
Cercopithecus	,,	...	50 ,,	(healthy)

These results were repeatedly confirmed by qualitative tests. Certain other diseases also tend to produce differences in the precipitum-forming power of the serum (p. 285).

It would be but seldom that such conditions could lead to error in forensic practice.

Fluid sera employed for qualitative measurements unless kept in

[1] The varying amount of precipitum obtained with the different bloods is expressed in %; the reaction given with homologous blood (human, in this case) being taken as 100 %. We are indebted to Dr Nuttall and Mr Strangeways for these (unpublished) figures.

sealed bulbs or closely stoppered bottles would be likely to become concentrated from evaporation, and give too high a reading.

Nuttall has also noticed that certain blood dried on filter-paper obtained from the tropics failed to go into solution and therefore gave no reaction.

Methods of diluting blood for the purpose of testing.

It has been noticed by many observers that solutions of fluid, or dried, sera in distilled water become cloudy, and that after 24 hours a precipitate occurs. In ·5 c.c. of a 1 in 21 dilution of human serum in distilled water this precipitate amounts to about ·001 c.c. We have, however, found that including this precipitate ·1 c.c. of human antiserum produces a smaller quantity of precipitum with blood diluted with distilled water than with the same specimen diluted with normal salt solution. The mean of three experiments in each case gave ·0384 c.c. of precipitum in salt solution dilutions and ·0328 c.c. in watery dilutions.

All observers are agreed that physiological salt solution (·6 %) is the best diluent, giving a clear solution which does not tend to cloud or deposit any material on standing.

Two opposite opinions, however, have been arrived at as to the influence on the reaction of increasing quantities of salt. Linossier and Lemoine (21. III. '02), taking 1 in 20 dilutions added increasing quantities of salt and found that even 1 % of salt impeded precipitation, and 5 % completely prevented it.

On the other hand Eisenberg (v. '02, p. 307) considers that even 18 % of salt has no influence, and Rostoski (1902, b. p. 42) found no noticeable difference with 10 %.

Our experiments agree more closely with those of the last observers. We have quantitatively estimated the influence of salt in the following way. Tubes containing 1 in 21 dilutions of human serum with gradually increasing percentages of salt were arranged in a rack, and to each ·1 c.c. of anti-human serum were added. We found that the precipita in the tubes containing the most salt were more flocculent, and owing to the increased specific gravity of the medium took longer to settle (Plate XI., fig. 2).

Results of measurements showed a slight decrease to 7 % and later an increase, probably due to the fact that the more flocculent precipitum, though really less in amount, occupies a greater volume.

Results of increasing quantities of salt in human serum dilutions.

Percentage of salt	Precipitum c.c.	Percentage of precipitum as compared with ·6 % salt solution	Percentage of salt	Precipitum c.c.	Percentage of precipitum as compared with ·6 % salt solution
·6 %	·0643	100 %	8 %	·0693	107·7 %
1 ,,	·0571	88·8 ,,	9 ,,	·0673	104·6 ,,
2 ,,	·0638	99·2 ,,	10 ,,	·0770	119·7 ,,
3 ,,	·0554	86·1 ,,	12 ,,	·0686	106·6 ,,
4 ,,	·0639	99·2 ,,	14 ,,	·0737	114·6 ,,
5 ,,	·0618	96·1 ,,	16 ,,	·0730	113·5 ,,
6 ,,	·0635	98·7 ,,	18 ,,	·0821	127·6 ,,
7 ,,	·0630	97·9 ,,	Saturated solution	·0854	132·8 ,,

Experiments with sheep and anti-sheep sera, which form more compact precipita, show the diminution in volume plainly. In this experiment the tubes were all centrifugalised for the same length of time in order to diminish the error due to the increasing specific gravity of the solutions.

Similar experiments to above with sheep serum.

Salt	Precipitum c.c.	Percentage of precipitum
1 %	·0199	100 %
2 ,,	·0203	102 ,,
4 ,,	·0201	101 ,,
10 ,,	·0140	70·3 ,,
Saturated solution	·0122	61·3 ,,

Qualitative estimations showed that when the quantity of salt was increased above 5 % the antiserum did not sink to the bottom, and that clouding occurred at the top of the tubes, and also took longer in forming.

The strength of dilutions used in quantitative and qualitative tests.

Strangeways, working in this laboratory, has found that dilutions of serum less than 1 in 10 do not give proportionately as much precipitum as lower dilutions. He considers that in such dilutions the precipita are apt to be redissolved, and states that they are more flocculent, and do not settle well owing to the high specific gravity of the fluid. He has ascertained by experiment that dilutions of 1 in 15 to 1 in 30 give proportionately larger quantities of precipitum which settle sooner and more compactly. We have worked throughout in quantitative observations with dilutions of 1 in 21, which are convenient to make and give very satisfactory results.

18—2

In most cases it would be impossible to estimate the strength of extracts from dry materials, but generally this must be rather low. Uhlenhuth (25. VII. '01) employs as controls dilutions of blood dried on glass for known periods, and matches as far as possible the tint of the control to that of the extract under examination. By means of a series of bloods of various kinds dried at different dates he is able to procure a control of about the same age as the material he is investigating.

The influence of temperature on the formation of precipitum.

Many authorities have published observations on this subject. Myers (14. VII. 1900) stated that the reaction took place rapidly at 37° C. Wassermann and Schütze (18. II. '01) and Michaëlis (9. X. '02, p. 734) confirm this observation, and Stockis (V. 1901) considers that 40°—42° C. is most favourable. Biondi (1902, p. 16) found the temperature to exert a considerable influence, but Linossier and Lemoine (1902) obtained reactions from 0°—58° C. Kister and Wolff (18. XI. 1902) contrary to all other observers state that there is no special difference in the reactions at room temperature and at 37° C.

Strangeways, working quantitatively, has shown that although the precipitum falls more rapidly at room temperature than at that of the ice-chest, and faster still at 37° C., yet finally the quantity of precipitum formed in each case is the same.

The relation of human serum to other body fluids.

Some of the materials used in these experiments had been preserved by the addition of a trace of chloroform for some months. This fact may render the figures given somewhat too low in comparison with fresh human serum. Specific reactions were produced, however, by all, though the reaction of amniotic fluid was very slight.

Anti-human serum No. I. was much more powerful than No. II., which moreover had undergone putrefaction.

Material	Anti-human No. I.	Anti-human No. II.	Percentages from means of these two	Anti-ox	Normal rabbit
1. Fresh human serum (2 days)	·0291 c.c.	·0197 c.c.	100 %	—	—
2. Old ,, ,, (8 months)	·0272 ,,	·0187 ,,	93·8 ,,	—	—
3. Placental serum (8 months)	·0150 ,,	·0112 ,,	54·5 ,,	—	—
4. Pleuritic exudate (2 weeks)	·0065 ,,	·0084 ,,	30·3 ,,	—	—
5. Hydrocele fluid (9 months)	·0046 ,,	·0037 ,,	16·7 ,,	—	—
6. Fluid from ovarian cyst (9 months)	·0018 ,,	trace	6·1 ,,	—	—
7. Amniotic fluid (9 months)	·0009? ,,	trace	3· ,, ?	—	—

Most observers have noticed that other body fluids, normal and pathological, as for example, pleuritic and ascitic exudates, hydrocele, ovarian, spermatocele and seminal fluids, synovia, albuminous and menstrual urine react with human antiserum; and in addition Nuttall (VI. 1901) observed that slight cloudings resulted with normal urine, as well as with nasal and lachrymal secretions.

The influence of age on blood to be tested.

Several observations have already been made on this subject. Uhlenhuth (25. VII. '01) found that dried human blood-stains 6 to 12 years old reacted with human antiserum in one minute. Biondi (1902) obtained positive reactions with dried human blood-stains 10—15 years old, but not with a specimen 20 years old. Ziemke (1901), however, was able to identify blood-stains 25 years old.

In order to make observations on this question we obtained blood-stained material from Scotland Yard, the museum of which contains the largest collection of forensic specimens in this country. The samples we obtained and tested have been tabulated in two classes according to the articles on which the blood was dried. The first class consists of metal, and the second of fabrics and leathers.

Blood dried on metal.

The results of experiments on 17 samples covering a period of 30 years, are arranged according to age in the following table. The number after each specimen refers to the catalogue of the Scotland Yard museum, and a short description of each is given in the appendix.

The reactions of all were neutral.

The following table refers entirely to weapons which had been preserved from rusting by the application of oil to the surface of the metal. This process had caked the blood into black masses, making it frequently difficult to say whether the mass consisted of blood and oil or rust and oil. In the majority of cases however it was possible to make certain of scraping off some blood. The material thus obtained was extracted with distilled water, and subsequently an equal volume of 1.2% salt solution added to it. If necessary the solution was filtered through filter-paper, and tested in the way described.

Excellent results were obtained from these materials, and showed conclusively that the property of producing a precipitum with its appropriate antiserum is not lost by blood dried on metal even after 30 years have elapsed.

Instrument from which the blood was obtained	Age	Foam test*	Result with anti-human serum	Control serum	Remarks
1. Razor	5 months	good	marked cloud, 5 mins.	anti-turtle nil	This had not been preserved with oil.
2. Knife	few months	,,	,, ,,	,,	,,
3. Pocket-knife (554)	9 months	,,	marked cloud, 1 hour	anti-dog nil	,,
4. Razor (550)	10 ,,	,,	,, ,,	,,	,,
5. Hatchet (539)	1 year	,,	cloud, 20 mins.	,,	,,
6. ,, (544)	1 ,,	,,	marked cloud, 1 hour	,,	Much oil. Blood obtained from a crevice in the hatchet.
7. Pocket-knife (530)	1½ ,,	,,	marked cloud, 10 mins.	,,	Thin layer of oil.
8. Dagger in sheath (524)	1½ ,,	,,	,, ,,	,,	No oil.
9. Two knives (525)	1½ ,,	,,	,, ,,	,,	Very little oil.
10. Knife (494)	3 years	slight	,, ,,	,,	Much oil. On first occasion no reaction. Instrument again scraped; reaction good.
11. Chopper, knives (491), and oil-can	5 ,,	,,	nil	,,	Owing to the amount of rust and oil present it was impossible to say whether any blood had been scraped off.
12. Razor (423)	6 ,,	good	cloud, 20 mins.	anti-turtle nil	
13. ,, (357)	10 ,,	,,	cloud, 45 mins.	,,	See Int, Table p. 272, No. 2.
14. Pocket-knife (547)	11 ,,	fair	slight cloud, 1 hour	anti-dog nil	
15. Knife (17)	28 ,,	slight	marked cloud, 15 mins.	,,	Tried also with very strong anti-deer serum to see if any ruminant blood present; no reaction.
16. Razor (20)	28 ,,	,,	cloud, 15 mins.	,,	Much blood and oil.
17. ,, (19)	30 ,,	,,	marked cloud, 3 mins.	,,	Thickly smeared with blood; little oil.

* "Foam-test" refers to whether or no the blood dilution foamed. As Nuttall has found, it is a valuable aid to determining that blood has gone into solution.

In one case, No. 11, however, no reaction was obtained; the negative result was probably due to little or no blood being present on that part of the knife which was examined. The condition of the weapon was such that it was impossible to be certain that the material scraped from it was blood, but it was thought better to include it in the series, so as to point out the possibility of a mistake occurring under such circumstances.

It appears that the effect of oil on blood is to lessen the reaction. This is probably due to the blood being coated with a film of oil, and therefore not so easily passing into solution.

Blood dried on organic materials.

The experiments quoted below have been inserted here to show the effects of age on blood dried on organic fabrics, but further experiments (p. 287) indicate some of the fallacies which may arise from the character of the materials. It happened, however, that in the specimens chosen few were of such a character as to give rise to possibilities of error.

The blood-stained materials tabulated below were all obtained from Scotland Yard, and with them two series of tests were conducted, the antiserum employed in the second being more powerful than that in the first.

In the first series very small quantities were employed, but in the second the amount in each case was slightly greater. It was, however, not found possible on either occasion to obtain more than very small fragments, and, moreover, none of the specimens, with the exception of No. 11, were markedly encrusted with blood. The one exception, a specimen of hair, 28 years old, was in some parts thickly plastered, and gave well-marked reactions with each antiserum.

The following table shows that numbers 1, 2, 3, 4, 6, 8, and 11, or, 64 % of the whole, gave well-marked reactions, their ages varying from 3 to 28 years. In No. 5 the paper was badly burnt and the capacity for reacting was probably destroyed by the heat. Nos. 7 and 10 produced alkaline solutions, and in each case the reaction with anti-human serum was very slight. This was probably due to the retarding influence of the alkali, which will be discussed later. At the time these experiments were carried out we were not aware of this action of alkalis. The negative result of No. 9 may have been due to its acidity. No. 12 failed to react, but we were unable to discover any reason for this. The controls in all cases were negative.

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	Material	Age	Foam test	Character of solution	Reaction to litmus	1st series		2nd series	
						Anti-human I.	Normal rabbit	Anti-human II.	Anti-ox
1.	Lining of clothes	3 years	good	clear	neutral	marked cloud, 60 mins.	nil		
2.	Felt hat	10 ,,	,,	(cloudy, clear (after filtering) clear	,,			cloud, 15 mins.	nil
3.	Printed paper	11 ,,	,,	clear	alkaline	cloud, 5 mins.	nil	cloud, 60 mins.	,,
4.	Part of same paper	11 ,,	,,	,,	neutral			cloud, 15 mins.	,,
5.	Same scorched	11 ,,	,,	,,	,,	nil	,,	nil	,,
6.	Alpaca dress	11 ,,	,,	,,	,,	immediate cloud	,,	marked cloud, 10 mins.	,,
7.	Braid	11 ,,	,,	,,	alkaline	slight cloud, 60 mins.	?cloud	slight cloud, 60 mins.	,,
8.	Cardigan jacket	11 ,,	fair	(cloudy, clear (after filtering) clear	,,		nil	marked cloud, 30 mins.	,,
9.	" Black Rep."	11 ,,	slight	clear	slightly acid	slight cloud, 60 mins.		slight cloud, 15 mins.	,,
10.	Cotton fabric, apparently washed	11 ,,	fair	,,	alkaline	slight cloud, 5 mins. no increase	,,		
11.	Hair	28 ,,	good	,,	neutral	cloud, 30 mins.	,,	marked cloud, 5 mins.	,,
12.	Wooden handle of chopper	28 ,,	none	,,	,,			nil	,,

As so little material was available the results may be looked upon as most satisfactory, for it can scarcely be doubted that more distinct reactions would have been obtained had it been possible to make more extensive use of the specimens.

Dried and Fluid Sera preserved in the Laboratory.

Experiments undertaken with 10 specimens of human blood preserved on filter-paper in the laboratory of ages ranging between two years and two days showed by the qualitative method no obvious differences, either in the rate or degree of clouding, on the addition of anti-human serum. Controls with anti-ox and anti-sheep sera were negative.

A few quantitative experiments quoted below made on fluid sera, preserved by sealing in glass bulbs, indicate that such sera lose their strength to some extent, though differences exist in the rate at which this occurs.

	Anti-human No. I.	Anti-human No. II.	Per-centage	Anti-ox	Normal rabbit
Human serum (1 week)	·0291 c.c.	·0197 c.c.	100 %	—	—
,, ,, (9 months) ...	. ·0272 ,,	·0187 ,,	93 ,,	—	—
	Anti-ox			Anti-human	
Ox serum (mean of 8 exps., p. 264) sealed 1 year	·0233 c.c.		100 %	—	
,, ,, (mean of 3 exps.) sealed 2 years	·0239 ,,		102 ,,	—	
	Anti-fowl's egg, No. I.	Anti-fowl's egg, No. II.			
Fowl's egg albumen (2 days) ..	·0254 c.c.	·0162 c.c.	100 %	—	
,, ,, (9 months) ...	·0160 ,,	·0112 ,,	67 ,,	—	
,, ,, (14 ,,) ...	·0225 ,,	·0144 ,,	88 ,,	—	

Antidiphtherial horse serum four years and six months old preserved with trikresol was found to produce a good but somewhat flocculent specific precipitum amounting to ·0572 c.c.

In the above experiments anti-human serum No. I. was only a few days old, whereas No. II. was three and a half months old, and was moreover contaminated by bacterial growths. The first anti-fowl's egg serum was quite fresh and the second three months old.

All sera of the same kind do not give with the same antiserum identical precipita, nor even the sera of the same individual at different times in some cases, consequently an accurate determination of the influence of age is not possible. Our experiments however seem to point to a slight decrease in strength as the result of age, the human

serum and fowl's albumen experiments showing a decrease of precipitum of 7 °/₀ and 12 °/₀ after 9 and 14 months respectively. The fowl's albumen kept for 9 months shows a decrease of 33 °/₀. It is, however, by no means easy to get accurate dilutions of egg albumen, and the relative weakness of the specimen may be due to this cause.

The two experiments just quoted also indicate that antisera lose some of their power, but not to the extent that some observers have stated. Some undoubtedly preserve their power of producing specific reactions after the lapse of 12 months. Others lose this property more rapidly, whilst some, as Nuttall has also found, become untrustworthy after a time, giving cloudings with all sera.

In considering the general results of these tables it appears that in the case of dried bloods time *per se* does not destroy their capacity for reacting with their own antisera. Judging from the control experiments with recently dried bloods we should think that the period between the addition of the antiserum and the formation of the cloud was increased, and the magnitude of the cloud diminished.

Fluid sera appear to deteriorate at any rate in some cases by keeping. It has been occasionally observed, however, in qualitative tests that old sera appear to react better than fresh ones.

The influence of putrefaction on sera and antisera.

Several observers have noted that blood even after putrefaction retains its power of forming a precipitum with its homologous antiserum. Uhlenhuth (1901), Nuttall (1901), and Biondi (1902), all obtained good reactions with putrid blood. Following a suggestion of Dr Nuttall's, in order to determine the influence of specific bacteria on serum, 1 in 21 dilutions in salt solution of ox and horse serum were inoculated with a series of organisms. Undiluted human pleuritic exudate was similarly treated. All were incubated for 5 days at 37° C. and then left at room temperature for 36, 50, and 40 days respectively; but the horse serum was allowed to undergo natural putrefaction also for the last 10 days. With the exception of the putrefactive bacteria none gave rise to very considerable growth, and in nearly all cases by the time of examination the organisms had sunk to the bottom, leaving the supernatant fluid clear. When necessary the fluids were filtered through filter-paper. All were slightly alkaline or neutral in reaction.

	Human pleuritic exudate (1—11)			Ox serum (1—21)			Horse serum (1—21) (contaminated)		
	Anti-human c.c.	%	Control anti-ox	Anti-ox c.c.	%	Control anti-human	Anti-horse c.c.	%	Control normal rabbit
Control. No organisms	·0234	100	—	·0173	100	—	·0572	100	—
Putrefactive organism } No. I.	·0280	119·6	—	·0140	80·9	—	·0713	124·4	—
,, No. II.	·0280	119·6	—	·0112	64·7	—	·0525	91·7	—
,, No. III.	·0280	119·6	—	—	—	—	—	—	—
Streptococcus	—	—	—	·0163	94·2	—	—	—	—
Putrefactive organism } No. IV.	·0215	91·8	—	·0163	94·2	—	·0666	116·4	—
B. anthracis	·0206	87·8	—	·0150	86·7	—	·0591	103·3	—
Hofmann's bacillus	·0187	87·8	—	—	—	—	—	—	—
B. subtilis	·0187	80·0	—	—	—	—	—	—	—
B. typhi	·0187	80	—	·0140	80·9	—	·0657	114·8	—
B. diphtheriae	·0187	80	—	—	—	—	·0670	117·1	—
Putrefactive organism } No. V.	·0187	80	—	·0084	48·7	—	·0582	101·1	—
Staphylococcus albus	·0187	80	—	·0169	97·6	—	·0754	111·5	—
B. coli	—	—	—	·0131	75·7	—	—	—	—
V. of cholera	—	—	—	·0112	64·7	—	·0670	117·1	—

In considering the above table in detail it is seen that the effects of various organisms on ox and human serum agree fairly closely with a few exceptions. The most striking are the putrefactive organisms I, II, and V. These differences may be due to the fact that growth in nearly all cases was less marked in the undiluted human, than in the diluted ox serum, the latter moreover was a year old and had been preserved in sealed tubes after filtration through porcelain. The effects on horse serum of the action of specific organisms combined with general putrefaction for 10 days agree with those of putrefactive organisms I, II, and III, on ox serum, in that the capacity for forming precipitum is increased.

It appears then from the few quantitative experiments we have made that the results of bacterial growth on sera differ, some reducing the quantity of precipitum produced and others raising it, neither action being however very marked. Such slight changes as do occur do not alter the specific character of the reaction.

Experiments were also made on human and other sera which had undergone natural putrefaction. Most of the materials had been kept for some time and consequently show the combined results of age and putrefaction.

	Material			Anti-human No. I.	Anti-human No. II.	Per-centage	Anti-ox	Normal rabbit
1.	Fresh	human serum	(2 days)	·0291 c.c.	·0197 c.c.	100	—	—
2.	Old	,, ,,	(9 months)	·0272 ,,	·0187 ,,	93·8	—	—
3.	Putrid	,, ,,	(5 months)	·0262 ,,	·0169 ,,	88·1	—	—
4.	Putrid	,, ,,	(8 months)	·0150 ,,	·0140 ,,	59·4	—	—
5.	Putrid	,, ,,	(9 months)	·0131 ,,	·0150 ,,	57·4	—	—
6.	Putrid placental ,,		(9 months)	·0150 ,,	·0112 ,,	53·7	—	—
					Anti-ox		Anti-human	
7.	Ox serum	(1 year old) mean of 8 exps.			·0233 c.c.	100	—	
8.	Ox serum, putrid (,, ,,) mean of 3 ,,				·0233 ,,	100	—	

The above table shows that in some cases advanced natural putrefaction seems to exert little influence, for although the precipitum is decreased considerably in Nos. 4, 5, and 6, yet this is not the case in Nos. 3 and 8. All the specimens had been putrefying for the time given in each case. Though time may have influenced Nos. 4, 5, and 6, it is more probable that organisms whose growth deleteriously affected the serum were present.

Finally, from the few experiments we have done we are of the opinion that putrefaction to almost any extent does not affect the specific precipitum-forming body.

Since blood dried in small quantities does not undergo putrefaction to any appreciable extent this factor may be neglected in ordinary medico-legal work.

Experiments already quoted (p. 273) with contaminated anti-human and anti-fowl's albumen sera demonstrated that putrefaction in sealed tubes does not affect the antibody in them, as has also been found by Nuttall.

An experiment conducted on the same blood dilution with a normal and a contaminated sample of the same antiserum gave as a mean of four estimations in each case ·0433 c.c. and ·0436 c.c. of precipitum respectively.

Moreover putrid (filtered) sera when injected produce, as several of us have found, powerful and specific antisera, and Strangeways has shown that the power of antisera made with similar doses of fresh and putrid filtered sera is nearly identical.

The detection of blood in the presence of lime, mortar, and earth.

The wide distribution of these substances rendered it necessary to investigate their action on blood, since in medico-legal practice it might

often be necessary to test blood dried on, or mixed with, these materials.

Solutions of earthy salts, mortar, and lime of various strengths were made in salt solution and tested qualitatively with various antisera to determine their action on serum. These actions vary to some extent with the quantity of serum added. In the following table the quantity added was one drop, since this was the unit chosen for qualitative experiments.

In this and other tables the following symbols have been used :

C = coagulation.		**D** = large deposit after 24 hours.
+ = marked cloud—full reaction.		D = smaller ,, ,, ,,
+ = less marked cloud.		d = smaller ,, ,, ,,
× = medium cloud.		tr = trace of deposit.
✻ = slight cloud.		· = no result.
✻? = very slight cloud.		— = no reaction.

Dilutions		Lime	Mortar	Calcium chloride	Calcium phosphate	Chalk	Sodium phosphate	Plaster of Paris	Alum	Caustic soda	Caustic potash
Saturated solution [1]	30 mins.	✻	✻	×	✻	✻	✻	✻			
	24 hrs.	d	d	d	✻	d	d	✻			
									1—25		
1—10		✻	✻	✻?	✻?	·	✻	·	**+**	✻	✻
		✻	✻	✻	·	·	·	·	**D**	✻	✻
1—100		·	·	·	·	·	·	·	×	×	×
		·	·	·	·	·	·	·	×	×	×
1—1000		·	·	·	·	·	·	·	×	✻	✻
		·	·	·	·	·	·	·	×	·	·
1—10,000		·	·	·	·	·	·	·	✻	·	·
		·	·	·	·	·	·	·	·	·	·

The addition of serum to strong solutions of lime resulted in a general clouding, which later gave place to a dense cloud below, which would be hard to distinguish from a positive reaction. Mortar gave rise to a similar but smaller clouding. Calcium chloride and sodium and calcium phosphates caused cloudings in very strong solutions only. The actions of caustic soda and potash in certain solutions are very marked, and will be referred to again later. They are briefly mentioned here owing to their presence in earth.

At this point it should also be noted that strong lime and calcium solutions give rise on standing, even after filtration, to deposits of the salt at the bottom of the tube and a filmy layer on the surface.

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[1] Where saturated solutions are mentioned the dilutions are 1—10 etc. of these.

It was found, however, that the difficulty in testing due to the presence of lime in mortar, plaster, and earth, could generally be eliminated in the following way. Solutions of all of the above substances were allowed to stand till the excess had settled to the bottom. The supernatant fluid was then pipetted off and filtered. Carbon dioxide gas generated by the action of dilute hydrochloric acid on chalk, and washed by passing through distilled water, was next passed through the fluid and the latter again filtered to free it from the presence of the precipitated calcium carbonate. By this procedure clear filtrates could be obtained in most cases, which remained so for an indefinite period, and produced no cloudings on the addition of sera.

The following formula explains the reaction :

$$Ca\,(OH)_2 + CO_2 = Ca\,CO_3 + H_2O.$$
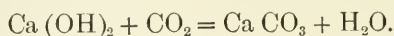
Too much of the gas must not however be passed into the solution owing to the fact that excess of CO_2 renders the insoluble carbonate again soluble :

$$Ca\,CO_3 + H_2O + CO_2 = Ca\,H_2\,(CO_3)_2.$$

By quantitative experiments it was found that though this process caused a deposition of blood pigment from blood solutions, yet the property of producing precipitation on the addition of appropriate antisera was not in any way affected.

The action of dry and wet lime, etc.

Lime was intimately mixed with human blood and then spread on porcelain and exposed to the action of air for three months. The resulting compound turned a greenish colour. Solutions of this gave an immediate clouding on the addition of serum. After the passage of CO_2, however, and subsequent filtration, no reaction could be obtained with anti-human or other serum. Under these conditions it seems that unslaked lime completely destroys the reacting power of blood in contact with it.

Quantitative experiments over a shorter period bring out the destructive quality of lime and mortar very markedly. To ascertain the action on serum of dry and wet lime, mortar, brick, earth, etc. weighed quantities of one gramme of each were mixed with 1 c.c. of human serum and allowed to act for 4 days. Similar mixtures but with 10 c.c. of water added were also prepared and allowed to stand for 4 days, to determine whether any different action was excited by these

materials in the presence of water.   At the end of this period all were made up to 1—21, by the addition in the former case of 20 c.c. of normal, and in the latter of 10 c.c. of double normal salt solution.

After applying the method of removing lime which has just been described quantitative estimations were made.

	Material		Anti-human	Anti-ox	%		Material		Anti-human	Anti-ox	%
1.	Control		·0403	—	100	6.	Earth	dry	·0244	tr	61
2.	Chalk	dry	·0367	tr	91			wet	·0291	tr	72
		wet	·0357	tr	89	7.	White brick	dry	·0262	tr	65
3.	Red brick	dry	·0281	tr	70						
		wet	·0347	tr	86	8.	Mortar	dry	tr	tr	0
4.	Pasteur filter	dry	·0309	tr	77			wet	·0028	tr	7
		wet	·0319	tr	79	9.	Lime	dry	tr	tr	0
5.	Berkefeld filter	dry	·0291	tr	72			wet	tr	tr	0
		wet	·0291	tr	72						

The above table shows that all the materials used in this series produced slight effects on the serum, but that mortar and lime completely destroyed its power of reacting.   All had been ground up very finely before the addition of the serum.

The effects of the lime present in ordinary earths.

The next point of importance was to determine whether the amount of lime present in ordinary earths was sufficient to interfere to any serious extent with the reaction.   For this purpose 9 samples of analysed earth were obtained, five from a field of gravelly land near Trowse, divided into five plots, and the others from different localities. The results of the analyses of these earths dried at 100° C. are given in the following table, arranged according to the percentage of lime present.   We are indebted to Mr T. B. Wood for these analysed earths.

	I. Trowse, plot 1	II. Trowse, plot 2	III. Trowse, plot 3	IV. Trowse, plot 4	V. Wryde clay	VI. Needham salt	VII. Trowse, plot 5	VIII. Bentwick fen	IX. Littleport fen
Total lime	·91	1·01	1·43	1·46	1·48	1·57	1·97	2·95	4·39
Organic matter, loss by ignition	5·62	5·16	5·27	5·52	14·48	5·40	6·31	39·35	50·82
Calcium carbonate	1·16	1·39	1·94	1·98	—	—	3·06	—	—
Total phosphoric acid	·16	·18	·16	·18	·37	·19	·20	·30	·28
Total potash	·13	·14	·13	·14	1·32	·31	·13	·63	·56
Total nitrogen	·15	·11	·14	·53	·24	·24	·13	1·42	1·8

Strong solutions of the above after simple filtration were at first clear but showed a white filmy deposit after standing.   The quantity of

this increased with the percentage of the lime present. On the addition of anti-human serum a thin cloud spread through the entire solution and gradually deepened, being considerably denser in No. IX than in No. I.

After the passage of CO_2 and filtration every solution was clear, with the exception of V and VIII, and produced no deposit on standing. The two mentioned were opalescent. No clouding occurred on the addition of anti-human serum.

Two sets of quantitative experiments were carried out with these soils. In the first 1 c.c. of finely divided soil was placed in a test-tube with 1 c.c. of human pleuritic exudate and 5 c.c. of water. After 4 days 5 c.c. of double normal salt solution were added to each, making a dilution of 1 in 11 of pleuritic exudate. In the second series 1 c.c. of dry earth was allowed to act for 4 days on 1 c.c. of pleuritic exudate. At the end of this period the specimens were diluted to 1 in 11 with salt solution.

These solutions were treated with CO_2 as described, and the precipita measured quantitatively.

Earth solution	Percentage of lime	Quantity of precipitum mean of the two observations	Percentage	Control anti-ox
No. I.	·91	·0404	100	—
No. II.	1·01	·0394	97·5	—
No. III.	1·43	·0319	78·9	—
No. IV.	1·46	·0389	96·2	—
No. V.	1·48	·0241	59·6	—
No. VI.	1·57	·0258	63·3	—
No. VII.	1·97	·0389	96·2	—
No. VIII.	2·95	·0314	77·7	—
No. IX.	4·39	·0383	94·8	—

The above table shows that the quantity of precipitum obtained did not decrease in proportion to the increase of the lime, which was apparently never present in sufficient quantity to materially affect the reaction. Excess of potash probably accounts for the low figures obtained in No. V, and possibly No. VIII (p. 284). In neither of these could a clear solution be obtained. Whatever may be the cause of the variations in the quantity of precipitum obtained, these experiments go to show that blood mixed with ordinary earth can be readily detected if present in sufficient quantity and that its specific character remains unaltered.

From our experiments on earth and lime salts we have drawn the following conclusions: (1) that the intimate mixture of lime with blood

completely destroys the latter; (2) that a clouding occurs in the solution of earth on the addition of serum; (3) that this is due principally to the presence of lime salts; (4) that the lime can be got rid of and the solution rendered clear, and not liable to clouding, by the passage of CO_2 and subsequent filtration; (5) that the passage of CO_2 in no way interferes with the reaction; (6) that the quantity of lime present in ordinary earth does not materially affect blood mixed with it.

The influence of chemical agents.

Observations on the reaction to litmus of extracts of coarse cloths and leather, as well as the possibility of the treatment of blood-stains in forensic practice with chemical reagents, made it desirable to investigate the action of such reagents on blood.

Acids.

Several experiments were made with dilutions of both organic and inorganic acids in distilled water and salt solution. Dilutions from 1 in 10 to 1 in 100,000 were tested by dropping in serum and noting the effects up to 2 hours and after standing for 24 hours. In these observations one drop of antiserum was added to about ·5 c.c. of the dilution since this has been the quantity uniformly used in qualitative work.

The addition of larger quantities produced slightly different results, probably owing to the alkalinity of the serum itself, and moreover, perhaps for the same reason, the effects of different sera were noticed to vary slightly. This remark applies to all the following experiments of a similar nature.

With the inorganic acids a noteworthy phenomenon was observed. Strong solutions (1 in 10) in salt solution caused coagulation of the serum and destruction of the precipitating substance, whereas weak solutions (1 in 100) produced no result. Dilution between 1—500 and 1 in 10,000 caused more or less clouding, in the latter case taking place half-way up the tube. These cloudings probably resulted from the precipitation of the albumen by the dilute acid and were observable within a few minutes. With greater dilutions nothing occurred. It was also found that neutralisation previously with sodium carbonate prevented these cloudings and in some cases even dissolved them after they had been formed.

One example is given below in detail.

Dilutions of Nitric Acid in normal salt solution	Anti-human serum		Anti-human serum after neutralisation	
	1 hour	24 hours	1 hour	24 hours
1—10	coagulation	coagulated mass	—	—
1—100	very faint cloud	cloud	—	—
1—1000	marked cloud	cloud	—	—
1—10,000	slight cloud half-way up tube	—	—	—
1—100,000	—	—	—	—

The precise degrees of clouding caused by the various dilutions differ with different acids.

Some organic acids behaved differently, acetic, oxalic, and tartaric causing little clouding in dilutions of 1 in 10, but marked cloudings in 1 in 100 and in 1—1000.

Details of the action of tartaric acid :—

Dilutions of Tartaric Acid in salt solution	Anti-human serum		Anti-human serum after neutralisation	
	1 hour	24 hours	1 hour	24 hours
1—10	very slight cloud	slight cloud	—	—
1—100	,, ,, ,,	medium cloud	—	—
1—1000	medium cloud	marked cloud	—	—
1—10,000	—	slight cloud half-way up tube	—	—
1—100,000	—	—	—	—

The accompanying table shows the chief actions of acid dilutions in salt solution on serum. These effects are more marked when the dilutions are made in distilled water.

		Sulphuric	Nitric	Hydrochloric	Acetic	Oxalic	Tartaric	Picric	Carbolic	Citric	Salicylic sat. sol.	Chinosol
1—10	30 mins.	C	C	+	·	※	※	·	C	·	+	·
	24 hrs.	**D**	**D**	**D**	·	※	※	·	**D**	·	**D**	·
1—100		·	※	·	※	※	※	C	※	※	※	※
		·	※	·	×	※	※	**D**	d	※	·	※
1—1000		※	×	×	※	×	×	+	·	×	·	※
		※	×	×	×	×	×	d	·	※	·	※
1—10,000		※	※	※	·	※	·	※	·	※	·	·
		·	·	·	·	·	·	·	·	·	·	·
1—100,000		·	·	·	·	·	·	·	·	·	·	·
		·	·	·	·	·	·	·	·	·	·	·

Strong alkalis and salts.

Experiments with the more powerful alkalis showed that in strong solutions cloudings were also produced in them on the addition of serum. Ziemke (17. VIII. 1901) has recommended the use of ·1 % caustic soda in distilled water for extracting blood-stains under certain conditions. Our observations show that in such dilutions cloudings are apt to occur on the addition of any antiserum, and render it thus an unsuitable agent for the process. These cloudings are better marked in dilutions in distilled water that with those in salt solution.

The effects of dilutions of caustic soda are given in detail below :—

Solutions of caustic soda in salt solution	Anti-human serum	
	1 hour	24 hours
1—10	slight cloud	slight cloud
1—100	medium cloud	medium cloud
1—1000	slight cloud	—
1—10,000	—	—
1—100,000	—	—

The following table shows the actions of dilutions of alkalis and salts on serum :—

		Caustic soda	Caustic potash	Sodium carbonate	Ammonia	Ammonium sulphate	Ammonium tartrate	Sodium & potassium tartrate	Sodium acetate	Potassium cyanide	Sodium citrate	Magnesium sulphate	Potassium nitrite	Potassium chlorate	Borax
										1—20				1—20	
1—10	30 mins.	✳	✳	·	·	✳	✳	✳	✳	✳	·	·	·	·	
	24 hrs.	✳	✳	·	·	✳	✳	·	✳	✳	·	·	·	·	
1—100		×	×	·	·	·	·	·	✳	✳	·	·	·	·	✳
		×	×	·	·	·	·	·	·	✳	·	·	·	·	·
1—1000		✳	✳	·	·	·	·	·	·	·	·	·	·	·	·
		·	·	·	·	·	·	·	·	·	·	·	·	·	·
1—10,000		·	·	·	·	·	·	·	·	·	·	·	·	·	·
		·	·	·	·	·	·	·	·	·	·	·	·	·	·
1—100,000		·	·	·	·	·	·	·	·	·	·	·	·	·	·
		·	·	·	·	·	·	·	·	·	·	·	·	·	·

Owing to the absence of any bad results from the addition of serum to sodium carbonate dilutions, we chose this reagent as being the most suitable for neutralising the effects of acids.

We next made some experiments to ascertain to what extent the acidity or alkalinity of the medium influenced the specific reaction. For this purpose both quantitative and qualitative experiments were conducted. The solutions in each case were made up in the following

way. Three series of 11 tubes were prepared, each containing ·5 c.c. of a 1 in 21 dilution of human serum in salt solution. To the first tube were added 5 drops of a solution of acid, to numbers 2, 3, 4 and 5, were added 4, 2, 3 and 1 drops of acid respectively. The sixth tube was not treated in any way. Numbers 7 to 11 received 1 to 5 drops of alkali respectively.

In the first series very small drops of a 1 in 100 dilution of hydrochloric acid was used, in the second series large drops of the same solution, and in the third series large drops of 1 in 10 solution of the same acid. Large drops of corresponding dilutions of sodium carbonate were used in series two and three.

In the quantitative experiments ·1 c.c. of anti-human serum was run into each tube, and in the qualitative one drop.

No. of tube		Drops	Series I. Small drops	%	Series II. Large drops	%	Series III.		
1.		5	·0309	(61)	·0009	(2)		nil	(0 %)
2.	1 in 100	4	·0319	(63)	·0018	(4)	1 in 10	,,	,,
3.	Hydrochloric	3	·0431	(85)	·0140	(27)	Hydrochloric	,,	,,
4.	Acid	2	·0431	(85)	·0187	(37)	Acid	,,	,,
5.		1	·0478	(93)	·0422	(83)		,,	,,
6.	Normal dilution of serum		·0507	(100)	·0510	(100)		·0516	(100 %)
7.					·0422	(83)		·0169	(31 ,,)
8.	1 in 100				·0441	(86)	1 in 10	·0150	(29 ,,)
9.	Sodium				·0422	(83)	Sodium	tr	?
10.	Carbonate				·0469	?	Carbonate	nil	(0 %)
11.					·0591	?		,,	,,

In series II the acidity and alkalinity varied from about 1—1000 to 1—5000 and in series III from about 1—100 to 1—500.

These experiments show that the presence of even small quantities of acid or alkali rapidly reduce the quantity of precipitum formed (see Plate XI., fig. 5). The apparent exceptions of numbers 10 and 11 of series II are due to the fact that the precipita produced were more flocculent and occupied more space than the more compact precipita elsewhere obtained. They also indicate that the presence of small quantities of acid or alkali do not alter the specificity of the reaction, for controls with anti-sheep serum were all negative.

Qualitative experiments undertaken on the same lines showed that with 1 in 100 solutions of acid and alkali, cloudings first occurred in the normal tube, next in the alkaline series, the times of their appearance increasing from No. 6 to 11. The last two showed faint traces of the specific reaction and also general opacity. On the acid side cloudings due to the acid rapidly appeared, but later specific cloudings were

superadded in Nos. 3 to 5. Control tubes tested with anti-ox serum showed general opacity in the last of the alkaline series and slight clouds in the acid series. Similar experiments with 1 in 10 solutions showed cloudings in the normal serum and first three specimens of the alkaline series only.

In the light of these observations it becomes necessary to test the reaction to litmus of all solutions which are to be examined and, if found decidedly acid or alkaline, to neutralise them.

It must also be remembered that the addition of strong acid or alkali to fluid or dried blood completely destroys it.

The effects of disease on the precipitum-forming power of serum.

Our experiments on this subject are only three in number but suggest that important differences may be found in diseased blood by means of this test. The following observations were made on sera from tuberculous cattle. The first required 2 c.c. of decinormal caustic soda per 100 c.c. of serum to give a pink tint with phenolphthalein, and the others 1·25 c.c. and ·8 c.c. respectively. Also the former required per 100 c.c. per 4·25 c.c. of decinormal caustic soda to produce a condition in which the serum was liquid when hot and solid when cold, and the latter 2 c.c. and 1·2 c.c. respectively. As a mean of three estimations in each case these sera produced ·0375 c.c., ·0328 c.c. and ·0244 c.c. of precipitum.

Sera	$\frac{NaOH}{10}$ per 100 c.c. to give pink with phenolphthalein	$\frac{NaOH}{10}$ required per 1000 c.c. to make serum liquid when hot, solid when cold	Precipitum
1.	2·0 c.c.	4·25 c.c.	·0375 c.c.
2.	1·25 ,,	2·0 ,,	·0328 ,,
3.	·8 ,,	1·2 ,,	·0244 ,,

Strangeways has made numerous observations (unpublished) on the differences in precipitum-forming power of the sera in disease.

The effects of antiseptics.

Lime, carbolic acid, and chinosol, might be taken under this heading but have already been discussed. We here propose to consider various volatile antiseptics, as well as such agents as formalin, mercuric perchloride, and copper sulphate, etc.

As an example of the important volatile antiseptics chloroform may be taken. In solutions containing much of this reagent on the addition of serum a white cloud, and later a deposit, occur. More dilute solutions give rise to slight cloudings. The results of experiments

with a series of such volatile antiseptics are given below. When only present in small quantities in preserved sera the possible error due to their presence can be eliminated by placing them in the incubator for half-an-hour to evaporate off the reagent. When present to a greater extent it was found that the supernatant serum above the deposit caused by them still retained its specific properties. This is in accord with what Nuttall has found.

		Corrosive sublimate 1—25	Copper sulphate	Formalin	Thymol	Chloroform	Alcohol	Benzol	Toluol	Xylol	Ether
1—10	30 mins.	C	C	**+**		×	C	×	✳	✳	✳
	24 hrs.	**D**	C	**D**		×	**D**	×	✳	✳	✳
1—100		×	C	✳	✳	✳	•	✳	✳	✳	•
		d	**D**	✳	✳	•	•	✳	✳	✳	✳
1—1000		✳	C	•	•	•	•	•	•	•	•
		✳	D	•	•	•	•	•	•	•	•
1—10,000		✳	+	•	•	•	•	•	•	•	•
		•	+	•	•	•	•	•	•	•	•
1—100,000		•	✳	•	•	•	•	•	•	•	•
		•	d	•	•	•	•	•	•	•	•

Corrosive sublimate and ferrous and copper sulphates were found to produce very marked effects. They apparently destroy the serum in contact with them and except when present in very small quantities it was found impossible to carry out the test. The effects of dilutions of corrosive sublimate and copper sulphate are given below.

Dilutions	Corrosive sublimate and anti-ox serum		Copper sulphate and anti-ox serum	
	1 hour	24 hours	1 hour	24 hours
1—25	immediate coagulation	large deposit	immediate coagulation	large deposit and cloud
1—100	dense cloud	,, ,,	,, ,,	,, ,,
1—500	cloud	cloud	coagulation and cloud	deposit & cloud
1—1000	,,	slight cloud	,, ,,	,, ,,
1—10,000	slight cloud	nil	marked cloud	,, ,,
1—100,000	nil	nil	slight cloud	small deposit

Silver nitrate causes an opaque white cloud on dilution with salt solution up to 1 in 10,000. Dilutions below this do not affect serum when added to them.

Formalin in 1 in 10 dilutions causes marked clouding, which increases till the whole contents of the tube are opaque white. Dilutions below 1 in 100 do not cause sufficient clouding to interfere

with the specific reaction. Solutions of thymol of 1 in 100 cause slight cloudings, but lower dilutions do not apparently affect sera.

Lysol and lysoform both cause great turbidity when added to salt solution even in low dilutions, and moreover even in very low clear dilutions the addition of serum causes clouding. No method has been devised for getting rid of these effects; consequently the presence of these substances except in very small quantities would render the test of doubtful value.

The effects of the reagents, which for the sake of convenience we have grouped under the heading of antiseptics, are very marked except in the case of the volatile class. Some of the latter when added in full strength to liquid sera produce heavy deposits, but the supernatant fluid retains its properties. Formalin and corrosive sublimate in strong solutions, as well as the sulphates of copper and iron and nitrate of silver in much weaker dilutions, completely destroy the precipitum-forming property. Lysol, lysoform, and similar antiseptics, owing to their property of forming cloudings with salt solution, render the application of the test of doubtful value in their presence.

The detection of blood dried on fabrics.

In order to determine to what extent the composition of different cloths influenced blood which had dried on them we procured a number of samples. Human blood was dropped upon these so as to leave some patches unaffected and others saturated. Subsequently the specimens were allowed to dry under natural conditions and were left undisturbed at room temperature and in the light for at least 30 days; some were not tested for nine months. First a series of control tests were carried out on unstained pieces of cloth in the following way. Small pieces 1 × 2 cms. were soaked overnight in 2 c.c. of distilled water. In the morning an equal quantity of double normal salt solution was added and the condition and reaction to litmus of the extract recorded. The majority of samples was found to be nearly neutral, some were distinctly alkaline, whilst most of the coarser materials were acid. About ·5 c.c. of each extract, if necessary after filtration, were placed in small test-tubes and 1 drop of serum added. No cloudings were noticed except in the markedly acid specimens. After neutralisation with sodium carbonate these also produced no effect on the serum. Certain solutions, especially the acid ones, were found to be opalescent, or slightly cloudy, before the addition of serum, but it was noticed that neutralisation tended to make these clearer. In all our experiments we have avoided shaking the extracts, as we frequently observed deposits and

Material		Solution	Reaction to litmus	Anti-human serum		Anti-ox serum	
				Immediate	6 hours	Immediate	6 hours
1. Black dress	(1 month)	clear	neutral	marked reaction	large deposit	—	—
2. Glacé silk	,,	,,	,,	,,	,,	—	—
3. Serge	,,	,,	,,	,,	,,	—	—
4. Green fancy serge	,,	green	,,	,,	,,	—	—
5. Sateen	,,	clear	,,	,,	,,	—	—
6. Merve	,,	,,	,,	,,	,,	—	—
7. Tweed cloth	,,	,,	,,	,,	,,	—	—
8. Furniture serge	,,	red	,,	,,	,,	—	—
9. Pillow case	(9 months)	clear	,,	,,	,,	—	—
10. Silk handkerchief	(1 month)	opalescent	,,	,,	,,	—	—
11. Blind ticking	,,	,,	,,	slight reaction	,,	—	—
12. Green velveteen	,,	green	alkaline	marked reaction	,,	—	—
13. Dark green cloth	,,	,,	slightly acid	,,	,,	—	—
14. Coarse flannel	,,	clear	acid	,,	,,	—	—
15. Felt hat	,,	,,	,,	medium reaction	medium deposit	—	—
16. Coarse duster	(9 months)	opalescent	,,	,,	,,	—	—
17. Brown canvas	(1 month)	,,	,,	,,	large deposit	—	—

When acid the solution was neutralised.

cloudy precipitates at the bottom of the tubes, which in some cases were very difficult to remove by filtration. After removing the supernatant fluid in solutions containing blood the tubes were, however, shaken to ascertain whether sufficient serum was in solution to produce marked foaming.

In testing for blood, stained patches were treated in the way described above and neutralised if necessary. Two small tubes of each solution were prepared. To one was added one drop of anti-human serum and to the other a drop of anti-ox serum. Two results of some of these experiments are given on the opposite page.

The detection of blood-stains on leather.

Several observations have been made with various samples of leather, which have been placed under a separate heading to more fully bring into prominence their peculiarities. It was found that nearly all gave acid reactions on solution. The degree of acidity, however, varied greatly, chamois leather being alkaline, suède kid glove only slightly acid, and the coarser leathers very decidedly acid. The addition of a drop of serum to the acid solutions produced clouding, and even coagulation with extracts of the coarser leathers. The latter also gave rise, especially if shaken, to bulky deposits in the original solutions.

Nearly all the solutions of leather could be neutralised and the blood-test satisfactorily employed. One class of leather was, however, a marked exception, namely, thick polished yellow leather. Solutions of this gave rise to extremely acid yellow fluids, whose colour deepened on the addition of alkali. It was found impossible to obtain the specific test for blood dried on it. At first it was thought possible that the blood was destroyed by the acid after solution, and extracts were made in alkaline salt solution to neutralise this effect. Even under these conditions no positive results could be obtained. Up to the present although many methods have been tried we have been unable to devise one which gives satisfactory results, and are forced to conclude that the mode of preparation of such leathers produces conditions which destroy the blood in contact with them. Under favourable conditions, when blood has been thickly deposited on the surface, it might, however, be possible to scrape it off and obtain a positive reaction. In the following table all solutions when necessary were neutralised, and filtered, before the addition of anti-human serum.

A series of experiments was also made to determine the effects of boot-blacking and polish. Blood-stains blackened over were hard to detect on the boot, but by neutralisation and filtration clear solutions

Material	Con-dition	Colour	Re-action	Anti-ox				Anti-human	
				Unneutralised		Neutralised		Neutralised	
				15 mins.	24 hours	15 mins.	24 hrs.	15 mins.	24 hours
Chamois leather	clear	clear	neutral	—	—	—	—	medium reaction	medium deposit
Suède kid glove	cloudy	,,	slightly acid	—	—	—	—	good reaction	large deposit
White ,, ,,	clear	yellowish	acid	slight cloud	slight cloud	—	—	,,	,,
Boot	,,	,,	,,	,,	slight deposit	—	—	,,	,,
Leather from in-side shoe	cloudy	,,	v. acid	,,	,,	—	—	,,	,,
Patent leather	,,	,,	,,	,,	,,	—	—	,,	,,
Yellow leather	,,	,,	,,	coagu-lation	deposit	cloud	cloud	cloud	cloud

could be obtained, and yielded well-marked reactions. Polish also made no difference to the test.

Experiments with saline solutions of tannin show that it has a very deleterious action on serum, rendering the application of the test when it is present in large quantities impossible. Solutions of 1 in 20 to 1 in 500 produce instant coagulation of the serum, and 1 in 1000 produces marked clouding.

Detection of blood on materials not previously mentioned.

Ten examples of wall paper of various textures and colours, red, brown, yellow, blue, and green, were tested and gave typical reactions. All produced neutral solutions, some of which were tinted.

Extracts of blood dried on various kinds of paper, stones, flint, slate, coal, cork, string, straw, rubber, linoleum, as well as silver and copper coins, yielded satisfactory results.

Although one piece of oak on which blood had been thickly incrusted gave a marked reaction with anti-human serum, we failed to obtain any reaction with blood on two blocks of cedar and pine. The quantity present on each of these was exceedingly small, and the negative result was probably due to this cause.

Foreign observers working with similar materials to some of those we have just enumerated were able to obtain in most cases satisfactory results.

These experiments demonstrate that many substances in common use give acid solutions. In most instances the acidity is not so marked as to be of importance, but in some unless recognised and neutralised might be liable to lead to grave error. Extracts of certain substances are sufficiently alkaline to impede the reaction.

1   2   3   4   5   6   7   8   9

Fig. 1.

1   2   3   4   5   6   7   8   9   10   11

Fig. 2.

1   2   3   4   5   6   7   8   9   10

11 12 13   14   15   16 17   18 19 20

Fig. 3.

PLATE 11

1 2 3 4 5 6 7 8 9 10

11 12 13 14 15 16 17 18 19 20

Fig. 4.

1 2 3 4 5 6 7 8 9 10 11

12 13 14 15 16 17 18 19 20 21 22
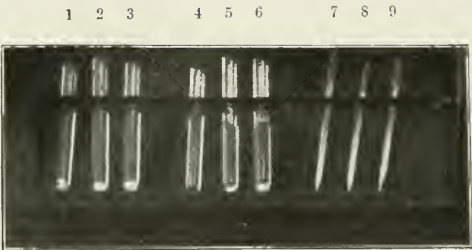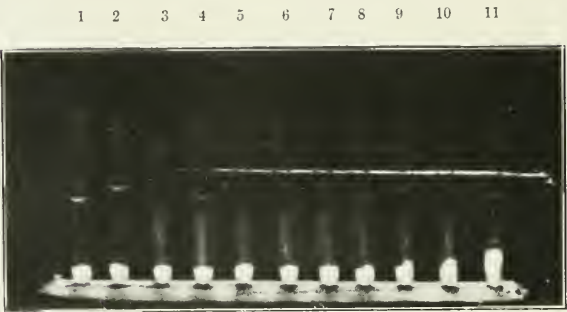
Fig. 5.

EXPLANATION OF PLATE XI.

Fig. 1. No. 1 shows the precipitum with normal human serum of (1—21 in salt solution) and anti-human serum (·1 c.c.). No. 2 with putrid human serum (1—21) and antiserum, and No. 3 with normal human serum (1—21) and putrid anti-human serum. No. 4 shows a clear solution of human serum in salt solution (1—21). No. 5 shows the deposit resulting from the solution of human serum in distilled water (1—21). No. 6 the precipitum formed with human serum diluted with distilled water (1—21) and anti-human serum. Nos. 7, 8 and 9 show three capillary tubes such as are used in quantitative measurements, and containing precipitum.

Fig. 2 Shows effects of increasing quantities of NaCl on the formation of precipitum; each tube contains ·5 c.c. of a 1 in 21 dilution of human serum, and ·1 c.c. of anti-human serum. No. 1 contains ·6% of salt, and those following 1%, 2%, 4%, 6%, 8%, 10%, 16%, 18%, and No. 11 is saturated with salt. Results of measurements are given on p. 267.

Fig. 3 Shows the specific precipitum in tests for human blood dried for a month on various materials. The lower series shows controls with anti-ox serum. The cloudings in the tubes are due to the opalescence of the solutions; and the various solid particles are portions of undescended precipitum.

No. 1 test for blood dried on silk handkerchief, No. 2 on tweed cloth, No. 3 on black dress fabric, No. 4 on dark green cloth, No. 5 on coarse green cloth, No. 6 on coarse red cloth, No. 7 on kid glove, No. 8 on blanket, No. 9 very coarse sack material, No. 10 on flannel. Nos. 11—20 show control tests with anti-ox serum: all negative.

The solutions whenever necessary were neutralised before testing.

Fig. 4. Nos. 1 to 5 show the effects on serum of dilutions of Hydrochloric acid in salt solutions of strengths of 1—10, 1—100, 1—1000, 1—10,000 and 1—100,000. No. 1 has a dense white cloud, No. 2 a slight cloud at the bottom, No. 3 a marked cloud, and the others are unaffected. Photographed after 6 hours.

Nos. 6—10 similarly illustrate the action of Tartaric acid, No. 1 (1—10) having a slight cloud, No. 2 (1—100) a medium cloud, No. 3 (1—1000) a marked cloud, Nos. 4 and 5 (1—10,000 and 1—100,000) are unaffected, the apparent deposit being due to the light.

Nos. 11—15 illustrate the action of Nitric acid. No. 1 (1—10) shows the coagulum, No. 2 (1—100) a very faint cloud, No. 3 (1—1000) a medium cloud, and Nos. 4 and 5 are unaffected.

Nos. 16—20 show the effects of Acetic acid. Nos. 1 and 2 (1—10 and 1—100) have slight clouds, No. 3 (1—1000) a medium cloud, and No. 4 (1—10,000) a marked cloud. No. 5 (1—100,000) is not affected.

Fig. 5 Illustrates the action of acids and alkalis on the formation of the specific precipitum. All the tubes contain ·5 c.c. of human serum dilution (1—21) in salt solution. Nos. 1—5 contain 5 to 1 drops of 1 in 10 Hydrochloric acid. No precipitates have been formed. No. 6 did not receive any acid or alkali. Nos. 7—11 contain 1—5 drops of 1 in 10 sodium carbonate solution; the quantity of precipitum shows a decrease along the series. Nos. 12—22 have been similarly treated but received drops of 1 in 100 acid and alkali respectively. The precipitum is seen to increase from 12 to 16 and decrease from 18 to 22. The slight clouding above the precipitum in each case is due to bacterial growth, the tubes having stood 48 hours.

We are indebted to Walter Mitchell, our laboratory attendant, for the time and attention he has bestowed on the photographing of these specimens.

IN MEMORIAM.

WALTER REED.

IT is with profound regret that we announce the death of Walter Reed, whose investigations into the etiology and prevention of yellow fever constitute one of the most valuable contributions to modern medicine.

In a letter to the Secretary of War of the United States, Professor Wm. H. Welch, of the Johns Hopkins University, stated that in his judgment, and in this all who are familiar with the subject must concur, Reed's researches form " the most valuable contributions to medicine and public hygiene which have ever been made in this country (America) with the exception of the discovery of anaesthesia. They have led and will lead to the saving of thousands of lives." His name will be remembered as that of a benefactor to mankind, for no discovery has yet been made in medicine which has been followed by such immediate and practical benefit.

Major Walter Reed, M.A., M.D., LL.D., Surgeon in the United States Army, was born in Gloucester County, Virginia (13 Sept. 1851), his father, the late Rev. Lemuel Sutton Reed, being a clergyman of the Methodist Church. His boyhood was spent in Virginia, and at an early age his tastes drew him to the study of medicine. After graduating as Doctor of Medicine from the University of Virginia he pursued his studies in Bellevue Hospital Medical College, New York, and in 1872 graduated from that Institution. He joined the U. S. Army Medical Department in 1875 [2,3]. In 1890-2 he was assigned to duty in Baltimore, in order that he might pursue research work in pathology and bacteriology under Professor Welch. In 1891 he published the results of a research upon the causes of hepatic lesions in typhoid fever. The writer was then Assistant in Hygiene and Bacteriology at the Johns Hopkins University, and the daily association with Reed in the

WALTER REED.

Born in Gloucester County, Virginia, 13 September, 1851.
Died in Washington, D.C., 23 November, 1902.

laboratory gave him an exceptional opportunity of judging his qualities. Suffice it to say that Reed's personality left an indelible impression on all of us with whom he associated. He was remarkably accurate and full of resolution in his work, and when he left us we were convinced that some day he would make his mark. In this he more than fulfilled our expectations.

Reed was next assigned to duty at St Paul, Minnesota, and subsequently was appointed bacteriologist to the office of the Surgeon-General and Curator of the Army Medical Museum at Washington. Here he developed the laboratories of pathology and bacteriology which have since become valuable departments of the Army Medical School. His time was mainly occupied with research. During the Spanish-American War he was made a member of a commission of medical officers " to investigate and report on the prevalence of typhoid fever in home camps," and it was at his instigation that the commission adopted a plan of collecting excreta in galvanized tanks which proved of considerable benefit, checking the disease at the Presidio, in California.

Turning his attention to yellow fever in 1900, being associated in his work with Major Carroll of his service, a sharp controversy ensued with Sanarelli, whose claim to the discovery of the *Bacillus icteroides* as the specific cause of yellow fever was denied. After the conclusion of the Spanish-American War, Reed was sent as president of a commission to study yellow fever at Havana. In view of the fundamental importance of the discoveries which followed, the Editors of the *Journal of Hygiene* subsequently requested Major Reed to send them a summary of the work done by the commission. His paper appeared in our second volume [1], and it is a source of gratification to the Editors to have since learnt from an official document [2] (p. 8) that "The history of the work is best given in Dr Reed's own words, in an article published in the *Journal of Hygiene*," the paper being republished *verbatim* in the document referred to. We can therefore but refer our readers to the paper we have cited.

Reed was the soul of the commission; in the words of General Leonard Wood [2], " His was the originating, directing, and controlling mind in this work, and the others were assistants only." And Professor Welch writes, " I am in a position to know that the credit for the original ideas embodied in this work belongs wholly to Major Reed." It seems necessary to quote these opinions in justice to Reed's memory for the reason that there has been a tendency in certain directions to

take from him the credit of discoveries which belongs to him and him only. Thus some would attribute the discovery of the part played by *Stegomyia fasciata* in the etiology of yellow fever to Dr Carlos Finlay of Havana, and it appears desirable to the writer to consider these claims so as to dispose of them in the manner they deserve.

To begin with, as far as I can ascertain from an exhaustive perusal of the literature of the subject, it was not Finlay, but Nott (1848) who appears to have been the first to attribute a part to insects in the dissemination of yellow fever. As stated in a monograph of mine published in 1899 [4], Nott did not claim the "insect theory" as his own, and he believed it applied also to malaria. Speaking of yellow fever, he dwells upon the fact that it occurs at times and places and under conditions favouring the development of insects; in other words, that the natural history of yellow fever is closely allied to the natural history of insects. He considered that yellow fever was most likely due to micro-organisms, "infusoria or animalcula," and that its spread from one locality to another is accomplished by the higher forms of insect life. Finlay (1881–1886) accused mosquitoes of playing the chief part in spreading yellow fever, stating that the limits of extension of the tropical mosquito, due to temperature, accounted for the geographical limitations of the disease. He considered that immunity to yellow fever might be produced by allowing mosquitoes which had sucked the blood of a yellow fever patient to subsequently bite the individual who was to be protected. He experimented with "*Culex cubensis*," and *Culex fasciatus* (known now as *Stegomyia fasciata*). In 1891 Finlay reported having subjected 67 persons to the bites of his supposedly infected mosquitoes. The experiments possess absolutely no weight, they are entirely unscientific. I shall not detail them here because I have always considered them worthless, they are described in detail in my monograph. *Finlay did not prove that any protection was afforded through the bites of infected mosquitoes, and he never attempted to show that these insects transmitted the disease.* It was natural that he stumbled on *Stegomyia fasciata*, a mosquito which has been all-too prevalent in Havana in the past. If he had been a scientific man he would have submitted his loose hypothesis to scientific tests, but he did nothing of the kind, though he has lived many years in Havana, where he has seen hundreds dying of yellow fever. Finlay advocated the mosquito-hypothesis for 19 years without supporting it by a single piece of evidence which could be accepted by men of science.

A vastly more suggestive observation was that of Carter of the

U. S. Marine Hospital Service, who studied the spread of yellow fever at Ormond, Miss. He showed that although the period of incubation of the disease was 5 days, 15 or 20 days had to elapse before a house became infected after a yellow fever patient had occupied it. This, combined with the demonstration by Ross and others of the part played by *Anopheles* in the etiology of malaria, led Reed to infer that the difference between the time of incubation and the time needed to infect a building was due to the fact that the infective agent passed through a stage of development in some insect host. He soon convinced General Wood, the military Governor of Cuba, with regard to the desirability of testing the theory experimentally, and he proceeded to do so in a manner worthy of modern science, being ably seconded by General Wood, to whom much credit is due for clear-sightedness.

If the writer has entered upon what may appear to be a matter of some controversy, it is due to the fact that he wishes to see justice done to the memory of Reed, whose dignified position in the matter placed him high above those who doubtless from party motives have striven to belittle his service to mankind. Unfortunately numerous Journals of lower order in America whose contributors are incapable of separating nonsense from scientific truth, have aided in belittling Reed's work, instead of being the first to give their countryman his due. It is doubtless for this reason that Reed failed during his lifetime to obtain official recognition of the great work he accomplished. We hope that the United States Government will do what it should toward aiding the widow of this distinguished officer, for he served his country well. At a memorial meeting recently held in Washington in Reed's memory, General Wood showed that he appreciated the magnitude of the service rendered when he said, "*I know of no other man on this side of the world who has done so much for humanity as Dr Reed. His discovery results in the saving of more lives annually than were lost in the Cuban War, and saves the commercial interests of the world a greater financial loss each year than the cost of the Cuban War*[1]. He came to Cuba at a time when one-third of the officers of my staff died of yellow fever, and we were discouraged at the failure of our efforts to control the disease. In the months when the disease was ordinarily worst the disease was checked and driven from Havana. That was the first time in nearly two hundred years that the city had been rid of it. The value of his discovery cannot be appreciated by persons who are not familiar with

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[1] The passage is not italicised in the original.

tropical countries.   Hereafter it will never be possible for yellow fever to gain such headway that quarantine will exist from the mouth of the Potomac to the mouth of the Rio Grande.   Future generations will appreciate fully the value of Dr Reed's services."

In an obituary notice which appeared in the *Virginia Medical Semi-Monthly* [3] it is stated that "There is good reason to believe that Dr Reed's health was severely shaken by the anxious experiences he had in investigating the cause and prevention of yellow fever, and he did not regain his former vigour up to the time that he was attacked by that dreaded disease, appendicitis, for which an operation was performed November 17, 1902.   He did not rally from the operation, and died November 23rd."

The writer in conclusion wishes to express the thanks of the Editors to Surgeon-General George M. Sternberg, U.S. Army, retired, as also to Major J. R. Kean, U.S. Army, for assistance in procuring data regarding Dr Reed, the last-named kindly sending us the excellent likeness which we have reproduced as a frontispiece to this number of the *Journal*.

G. H. F. N.

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(2)   The Scientific Work and Discoveries of the late Major Walter Reed, Surgeon in the Army of the United States.   Prepared by Major Jefferson Randolph Kean.   U. S. Senate, Document No. 118, 57th Congress, 2nd Session. Ordered to be printed, 28 Jan. 1903.   (Washington : Government Printing-Office, 1903.)

(3)   Life of Surgeon Walter Reed, U.S.A.—the Discoverer of the Cause of Yellow Fever and the Means of its Prevention.   *The Virginia Medical Semi-Monthly*, vol. VII., pp. 450–453.   (9 Jan. 1903.)

(4)   Nuttall, G. H. F. (Oct. 1899).   On the *rôle* of Insects, Arachnids and Myriapods, as Carriers in the spread of Bacterial and Parasitic Diseases of Man and Animals.   A critical and historical study.   *Johns Hopkins Hospital Reports*, vol. VIII., pp. 154, 3 plates.   (See especially pp. 25–27.)

THE GRAPHIC METHOD OF CONSTRUCTING A LIFE TABLE ILLUSTRATED BY THE BRIGHTON LIFE TABLE, 1891—1900.

By ARTHUR NEWSHOLME, M.D. (Lond.), F.R.C.P., and
T. H. C. STEVENSON, M.D. (Lond.), D.P.H. (Camb.).

Ten years ago one of us constructed a Life Table for Brighton[1], based on the experience of the ten years 1881—90. This Life Table was constructed by the graphic method originally employed by Milne in the construction of his famous Carlisle Table[2]; and the same method of construction is here adopted and described. Dr Hayward has given in this Journal[3] a description of the analytical methods of constructing a Life Table. In the present paper it is proposed to describe and discuss the graphic method of constructing a Life Table. Medical Officers of Health can then decide which method of construction they will adopt. Remarks on the comparative value of the two methods will be made at a later part of this paper. So far as practicable, an example will be given of the working at each stage, in order that the calculations may be followed with a minimum of trouble.

Data.

The data on which a Life Table is formed are the number and ages of the living and the number and ages of the dying. The ideal Life Table would represent " a generation of individuals passing through time " and measure the probabilities of life and death of this generation at birth, and of the survivors at each successive age until the whole generation became extinct. The experience thus watched would be obsolete before it was available. In practice therefore it is preferable to investigate the mortality experience of a population at various ages, from birth to the most advanced age during a recent period.

The present Life Table deals with the experience of Brighton in 1891—1900. The practice of the Registrar-General with regard to

the inclusion of deaths occurring in institutions within and exclusion of deaths without Brighton has been strictly followed. No deductions have been made for deaths of visitors occurring in private houses, boarding-houses, and hotels in the town.

The first step is to ascertain the populations in 1891 and 1901, and the deaths in the ten years 1891—1900. These are given in the following table :—

	Population of Brighton				Deaths in Brighton 1891—1900	
	Census 1891		Census 1901			
Age	Males	Females	Males	Females	Males	Females
0—	7189	7185	7226	7425	4368	3804
5—	7260	7290	7088	7185	237	257
10—	6940	7419	7031	7479	144	194
15—	5960	8729	6694	9305	232	229
20—	5035	9179	5891	9992	285	264
25—	9280	14,095	10,339	16,582	688	665
35—	7413	10,569	8888	12,454	966	928
45—	5412	7867	6630	9495	1197	1176
55—	3632	5528	4341	6537	1319	1415
65—	2293	3511	2533	3889	1509	1942
75—	787	1314	976	1650	1140	1790
85 & up.	92	229	122	261	293	591
	61,293	82,915	67,759	92,254		

Number of Years of Life in Age-Groups.

It is then necessary to ascertain the total years of life at risk in the ten years.

The following example illustrates the method of obtaining these for each age-period.

If P_1 is the male census population at ages 10—15 in 1891 and P_2 the same in 1901 and r the population resulting per unit in the ten years, then the total years of life at risk at age 10—15 $= \dfrac{P_1 \times r^{\frac{1}{10}} \times (r-1)}{r^{\frac{1}{10}} - 1}$.

$$r = \frac{P_2}{P_1}, \ P_1 = 6940, \text{ and } P_2 = 7031,$$

therefore $\qquad\qquad r = \frac{7031}{6940}.$

Therefore
$$\log r = \log 7031 - \log 6940$$
$$\log 7031 = 3\cdot8470171$$
$$\log 6940 = 3\cdot8413595$$
$$\log r = \cdot0056576$$
$$\log r^{\frac{1}{10}} = \tfrac{1}{10}\log r = \cdot00056576$$
$$\log r^{\frac{1}{40}} = \tfrac{1}{40}\log r = \cdot00014144$$
$$r = 1\cdot01311233$$
$$r^{\frac{1}{10}} = 1\cdot001303553.$$

From the above formula log. of total years of life at risk required

$$= \log P_1 + \log r^{\frac{1}{40}} + \log (r-1) - \log (r^{\frac{1}{10}} - 1).$$
$$\log P_1 = 3\cdot8413595$$
$$\log r^{\frac{1}{40}} = \cdot0001414$$
$$\log (r-1) = \overline{2}\cdot1176796$$
$$\overline{1\cdot9591805}$$
$$\log (r^{\frac{1}{10}} - 1) = \overline{3}\cdot1151286$$
$$\overline{4\cdot8440519} = \log \text{ of years of life required.}$$

Therefore total years of life at risk = 69,831.

N.B. To ensure accuracy the value of $r^{\frac{1}{10}}$ must be calculated by the use of eight figure logarithms (as given in the ordinary table-books for the numbers 10,000 to 10,800). Using seven figure logarithms in the above calculation the result obtained is 69,829.

By the use of the following method the same result is obtained in three stages :

1. The ratio of increase in the ten years is first found as before by the formula
$$P_2 = P_1 \times r,$$
$$7031 = 6940 \times r,$$
therefore
$$r = \tfrac{7031}{6940}.$$
Therefore
$$\log r = \log 7031 - \log 6940$$
$$\log 7031 = 3\cdot8470171$$
$$\log 6940 = 3\cdot8413595$$
$$\log r = \cdot0056576$$
and as before
$$\log r^{\frac{1}{10}} = \cdot00056576$$
and
$$r^{\frac{1}{10}} = 1\cdot001303553 = \text{population resulting per unit per annum.}$$

2. The central population of each census year (that is, the population at the middle of the year, three months after the assumed census date, Apr. 1) is then found by the formula $C = P \times r^{\frac{1}{40}}$.

Therefore
$$\log C_1 = \log P_1 + \tfrac{1}{40}\log r,$$
and
$$\log C_2 = \log P_2 + \tfrac{1}{40}\log r.$$
Therefore
$$\log C_1 = 3\cdot8413595 + \cdot0001414,$$
$$\text{or } 3\cdot8415009,$$
and
$$\log C_2 = 3\cdot8470171 + \cdot0001414,$$
$$\text{or } 3\cdot8471585.$$

Hence $C_1 = 6942\cdot260$ and $C_2 = 7033\cdot288$.

3. The total years of life at risk in the decennium are now found from the formula

$$\text{years of life at risk} = \frac{C_2 - C_1}{\text{annual increase per unit}} = \frac{91 \cdot 028}{r^{\frac{1}{10}} - 1},$$

therefore log of total years of life at risk required

$$= \log 91 \cdot 028 - \log \left(r^{\frac{1}{10}} - 1 \right).$$

$$\log 91 \cdot 028 = 1 \cdot 9591750$$

$$\log \left(r^{\frac{1}{10}} - 1 \right) = \log \cdot 001303553 = \overline{3} \cdot 1151286$$

$$\overline{}$$

$$4 \cdot 8440464$$

therefore years of life $= 69,831$.

Mr A. C. Waters has devised [1] a method of estimating the mean population of an intercensal period which reduces the actual calculation of estimated populations and therefore of years of life at risk into a very simple form. This method is an application of algebra to the method of uniformly changing proportions. He assumes that the whole population increases in geometrical progression at a constant rate, while the proportion of any selected part to the whole changes in arithmetical progression at a constant rate. These assumptions enable consistent estimates to be obtained, in which the summation of the parts is equal to the independent estimate of the population as a whole. Certain factors m and n are obtained in this method, based upon the rate of increase of the population of England and Wales, and these on application to the whole population of Brighton and to each age-group of that population give the mean population. This when multiplied by ten is the total number of years of life at risk.

The value of $m = \cdot 5445944$, and $\log m = \overline{1} \cdot 7360732$.

„ „ $n = \cdot 4564973$, and $\log n = \overline{1} \cdot 6594383$.

Thus for males aged 0—5

$$\text{Total years of life at risk}$$

$$= 10 \left(m P_1 + n P_2 \right)$$

$$= 10 \times \left(m \times 7189 + n \times 7226 \right).$$

$$\log m = \overline{1} \cdot 7360732$$

$$\log 7189 = 3 \cdot 8566685$$

$$\overline{}$$

$$3 \cdot 5927417,$$

therefore $\qquad 7189 \times m = 3915 \cdot 09.$

$$\log n = \overline{1} \cdot 6594383$$

$$\log 7226 = 3 \cdot 8588980$$

$$\overline{}$$

$$3 \cdot 5183363,$$

therefore $\qquad 7226 \times n = 3298 \cdot 65.$

Hence number of years of life at risk in 1891–1901

$$= (3915 \cdot 09 + 3298 \cdot 65) \times 10 = 72,137.$$

The following figures show the years of life of males at each age-group when calculated by the two methods:

Age	Years of life of Males calculated by		Difference
	Old method	Mr Waters's method	
0—	72,067	72,137	70
5—	71,781	71,894	113
10—	69,831	69,891	60
15—	63,016	63,016	0
20—	54,303	54,312	9
25—	97,725	97,735	10
35—	80,913	80,944	31
45—	59,699	59,739	40
55—	39,582	39,596	14
65—	24,050	24,051	1
75—	8,734	8,741	7
85 & upwards	1,055	1,058	3
	642,756	643,114	358

When calculated by means of the factors m and n the number of male years of life is 358 in excess, while the corresponding number of female lives is 428 in excess of the number calculated by the older method.

In the present Life Table the figures obtained by the older method have been used in order that the results for 1891—1900 may be comparable with those for 1881—90. The difference between the results obtained by the two methods is too small materially to affect the Life Table. For those compiling a Life Table for the first time, the use of the factors m and n is recommended, as it saves a considerable number of logarithmic calculations.

Number of Years of Life at Risk and Deaths in Single Years of Life.

Having now obtained a statement of the total number of years of life at risk in quinquennial and decennial groups of ages, the process by which the corresponding numbers for individual years of life have been obtained, must be examined. This, except for the first five years of life, has been done by an adaptation of the graphic method. The reasons for adopting this method, and for regarding it as preferable to the

analytical methods are for the sake of convenience given separately on page 314 *et seq.*

The method may be briefly described as follows: Along the abscissa line AZ (Plate XII.) mark off five equal portions, each to represent five years, for the first five quinquennial intervals of age; and let seven other equal portions, each of double length to represent ten years, succeed them for the subsequent decennial intervals of age.

At each of the points, A and B, erect perpendiculars to AZ, and make the perpendicular lines of such a height in accordance with the marginal scale previously decided upon that the parallelogram Ab shall equal in dimensions the population living aged 0—5. Thus in the diagram of the male years of life at risk, $Bb = 14413\cdot4$, and this when multiplied by 5, the number of years (0–5) included between A and B $= 72067$. Similarly $Cc = 14356\cdot2$, and this when multiplied by 5, gives 71781 as the area of $BbcC$. In the later groups 10 years are taken. Thus $Gg = 9772\cdot5$, the area of Fg being 97725. Having thus plotted out the populations living at various groups of ages, the number living at each single year of life is obtained in the following manner.

A curved line is described through the parallelograms already drawn, sweeping as easily as possible through the upper part of these parallelograms from A to Z. This curved line (1) must be as little curved as other conditions will admit of. (2) It must never change its direction abruptly so as to form an angle in its path. (3) The curved line thus described must so cut each of the parallelograms that the area included between the base line below, the corresponding portion of the two ordinates laterally, and the portion of the curved line above, shall equal the area of the parallelogram erected on the same base. Thus the area of the parallelogram $Cd =$ the area of $Cc'd'e'D$. In other words the area cut off must exactly equal the area added.

If now the distances AB, BC, CD, DE, &c., along the abscissa line be divided into equal portions representing one year each, vertical lines drawn from the centre of each of these spaces will give the central population for each year of age.

The accuracy of the curve is confirmed by ascertaining that the sum of the ordinates drawn from the base line within each space to the curved line bounding the space above is equal to the area of the parallelogram drawn on the same base. Thus in Plate XII., $De = 63016$ $=$ the sum of the five ordinates, $13350 + 13030 + 12670 + 12210 + 11750$.
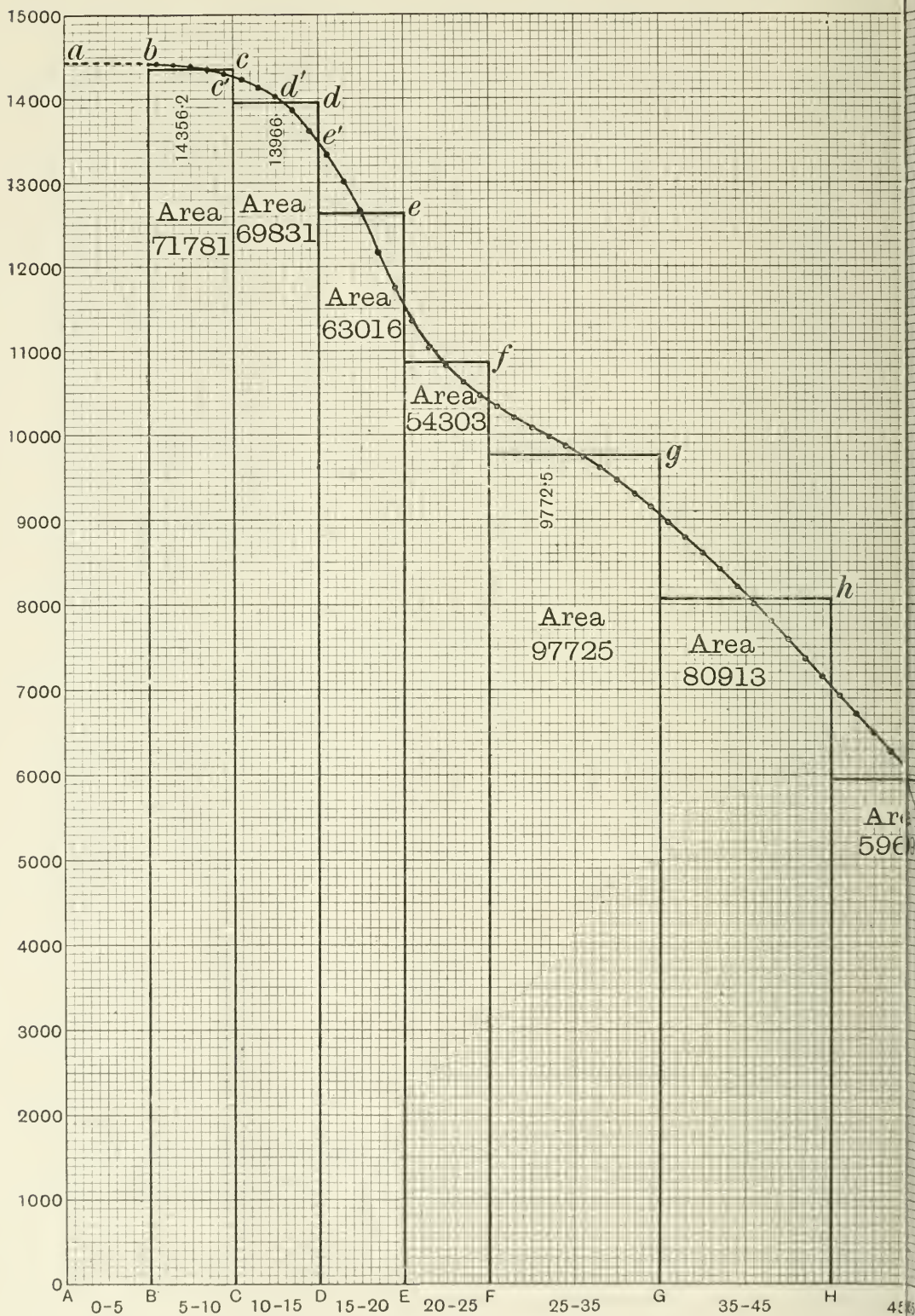
Note. The horizontal dotted line *ab* shewn above is not part of the

PLATE XII

—MALES.

Area
969

Area
39582

Area
24050

Area
8734

Area
1055

| 45 | 55–65 | 65–75 | 75–85 | 85 and upwards |
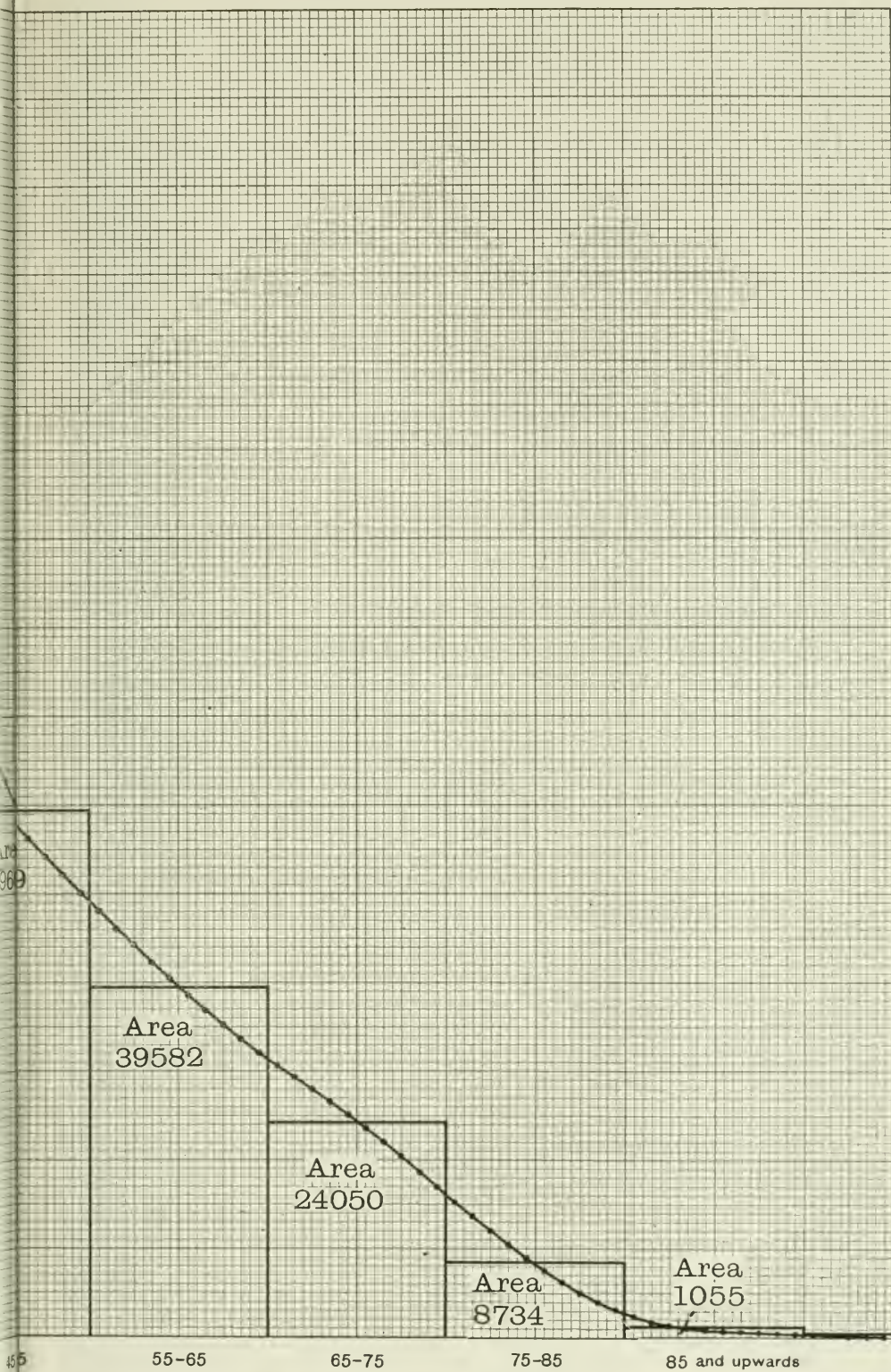
ve, but represents the upper extremity of the parallelogram Ab.

PLATE XIII

-MALES.

Area
1319

Area
1509

Area
1140

Area
293
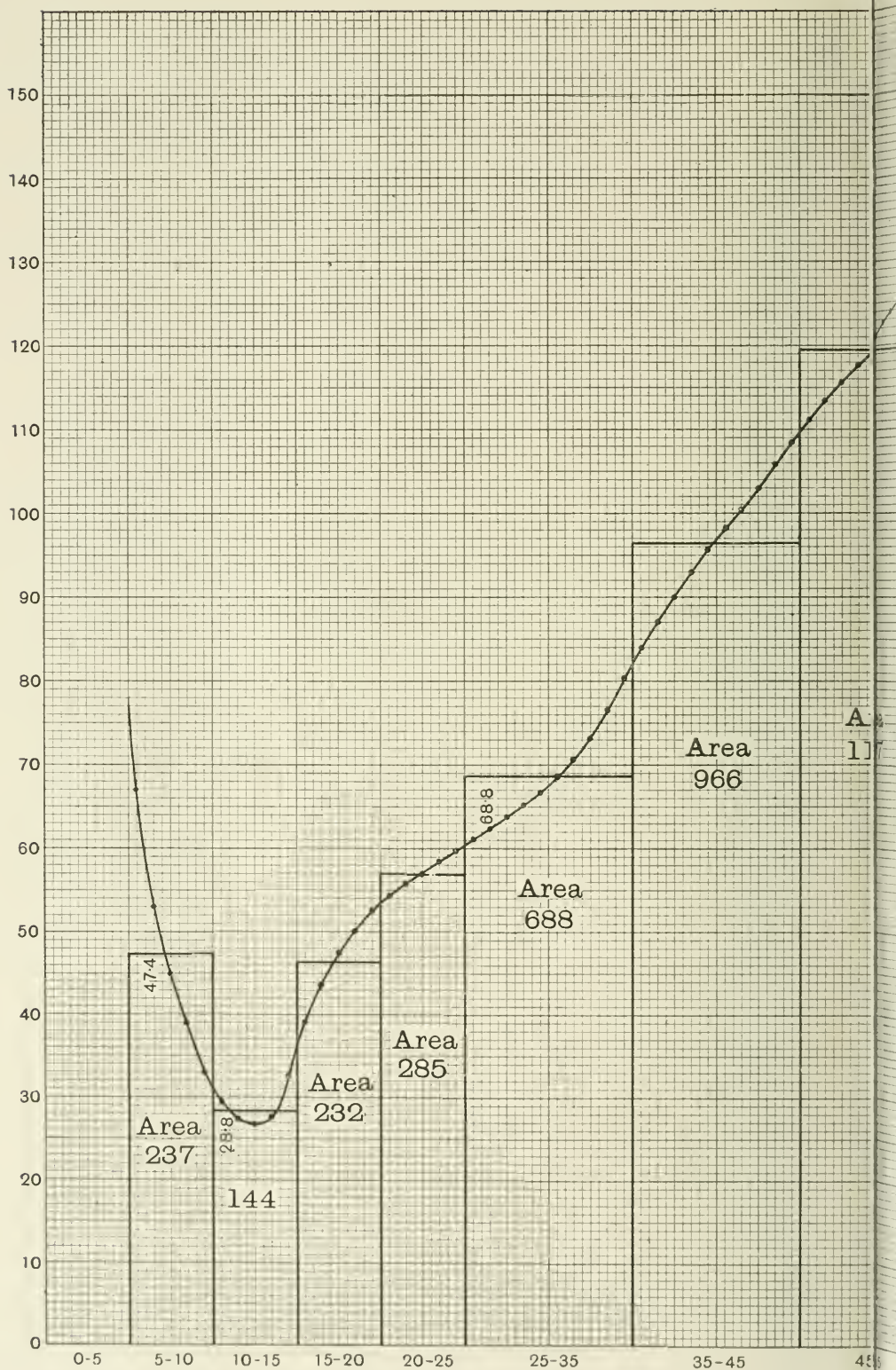
55-65   65-75   75-85   85 and upwards
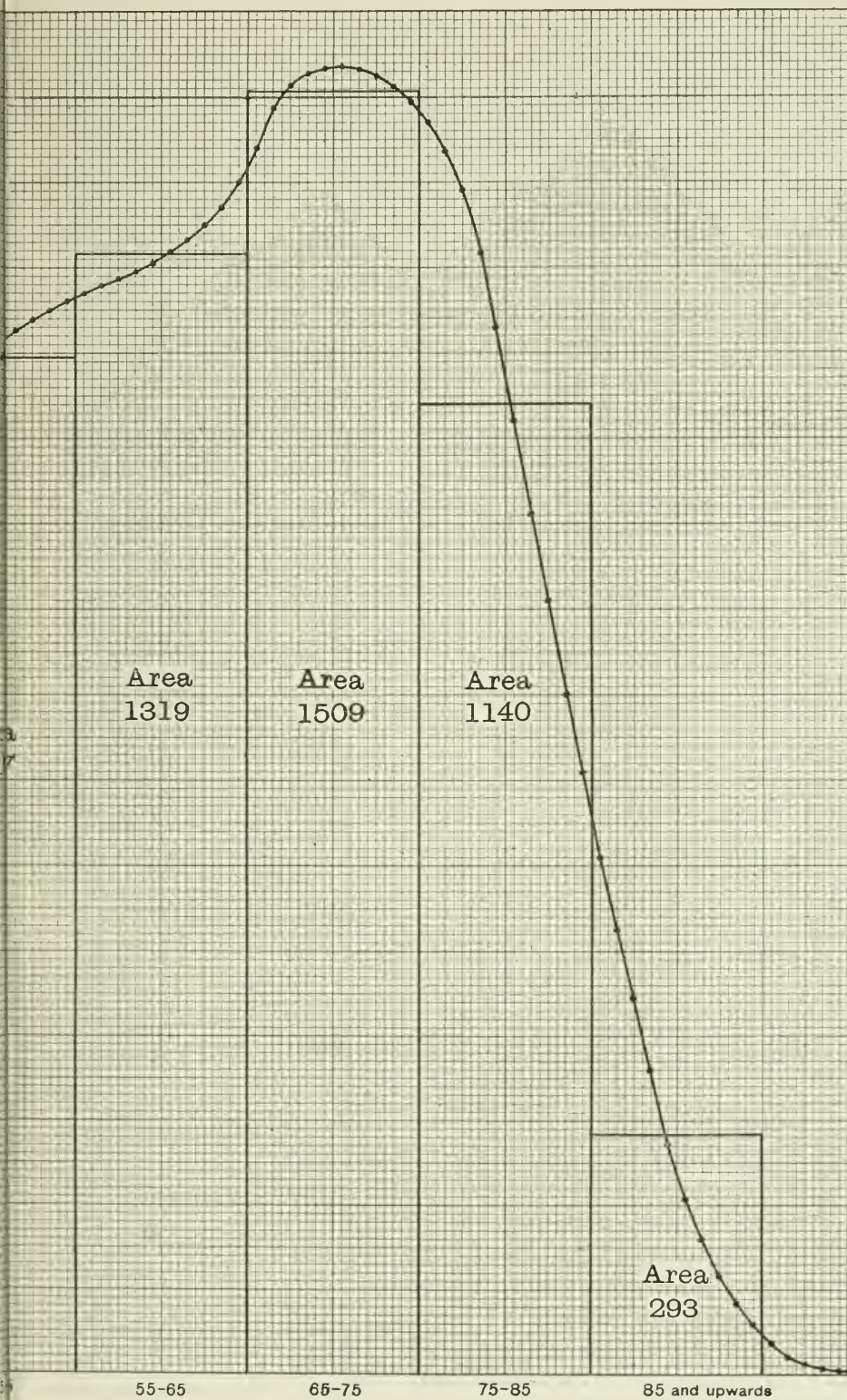
The scale adopted for the curve of lives at risk (Plate XII.) is $\frac{1}{8}'' = 100$, for the deaths (Plate XIII.) $1'' = 8$.   The plates as published are reduced to two-fifths of the size of the originals.   Experience shows that the errors due to defective measuring of ordinates are insignificant in their effect. A constant check upon such possible errors is maintained by the fact that the sum of the five or ten ordinates must equal the area of the parallelogram for the same age quinquennium or decennium.   Hence the error, if any occurs, is merely one of distribution among these 5 or 10 years of life, and affects only the p_x values for these ages.   Any possible slight gain or loss at one age is compensated for by a corresponding loss or gain at the immediately previous or successive ages.

If these rules are followed, it is improbable that any material discrepancy will arise when different draughtsmen deal with the same curve.

Having described in full the method by which the central population for each year of life is ascertained, it is unnecessary to describe the same process for the deaths occurring at groups of ages.   Plate XIII. dealing with male deaths shows the details of the process.   Thus for the deaths $5 - 10$, the total area is composed of $67{\cdot}0 + 53{\cdot}0 + 45{\cdot}0 + 39{\cdot}0 + 33{\cdot}0 = 5 \times 47{\cdot}4 = 237$.

It is unnecessary to reproduce here the table in which the results obtained as above are set forth.   The following extracts sufficiently explain the data.

Total Lives at Risk and Deaths for each Year of Age.   Males.

	Population		Deaths	
Age	In original groups	Distributed	In original groups	Distributed
5		14,400		67·0
6		14,380		53·0
7	71,781	14,370	237	45·0
8		14,340		39·0
9		14,291		33·0
10		14,205		30·5
11		14,115		27·5
12	69,831	14,000	144	27·0
13		13,870		27·0
14		13,661		32·0
75		1552		147·3
76		1388		141·0
77		1224		139·0
78		1050		131·7
79		900		123·0
80	8734	750	1140	112·0
81		620		101·0
82		520		91·0
83		410		80·0
84		320		71·0

Instead of separately obtaining the years of life at risk and the deaths for each age as shown above, it has been suggested that average p_x values should be calculated for the different age-groups by the formula (see p. 311)

$$p_x = \frac{2P_x - d_x}{2P_x + d_x},$$

and that these average p_x values should then be plotted out as parallelograms, a curve constructed as above described and values of p for each individual year of life read from the curve. But when p_x values for each year of life have been calculated a valuable check on the accuracy of the two curves upon which they are based is constituted by plotting out the p_x values in a curve. If this curve does not run smoothly, the facts as to the lives at risk or deaths in the corresponding years of life can be reconsidered; whereas if the p_x curve be drawn directly no such test can be applied[1]. Furthermore, p_x represents a factorial value, while P_x and d_x are entities. Hence the effect of diminishing or increasing the value of p varies according to the quantity to which it is applied, *i.e.* in the construction of the Life Table, the l_x column. It follows that as the value of l_x is constantly diminishing, a given amount added at ages 25—30 to the average value of p for ages 25—35 must be compensated for by a greater amount subtracted from the values of p at ages 30—35. This is shewn in Plate XIV., p. 310. Thus the rule for checking the accuracy of curves given on p. 302 is not applicable to a p_x curve.

Years of life at risk at ages 0—5.

The graphic method just described gives for a large community accurate results for the years of life from 5 to 85. The first five years of life present special difficulty whatever method of calculating the central population for each of these years of life is adopted. This arises from the fact that the extremely rapid changes in the rate of decrease of mortality at this age-period require very complete data for their exact statement; and the additional data furnished to meet this want by the census and death returns for each age 0—5 are found to be inaccurate. The ages of young children are often erroneously

[1] In our experience the p_x curve has only been found to be irregular to an extent suggesting the desirability of such reconsideration at one place, viz. at 65—75 in the female curve. On reference to the curves of population and deaths there was no difficulty in concluding that the death curve as originally constructed was too much truncated at its apex; and on readjustment a smooth p_x curve was obtained.

stated in the census returns. Hence, although the total number aged 0—5 may be accepted as accurate, the distribution of this total at each age under 5 must be found by an independent method.

Two methods have been adopted for this purpose. The first used by Dr Farr [5] was adopted in the Brighton Life Table 1881–90. The second was used in the Life Table for England and Wales for 1881–90. The first method is based on the births during the decennium. The number of children in any one calendar year reaching the exact age of one year may be taken as equal to the births from July 1st to December 31st of the preceding year *plus* the births from January 1st to June 30th in the same year, and *minus* the deaths under one year of age during the same year. Similarly the number of children reaching the age of one year for the ten years 1891–1900 may be taken to be equal to the total births 1890½ to 1899½, *i.e.*, from July 1st 1890 to June 30th 1900 *minus* the number of deaths under one year of age in the ten years, 1891–1900.

Thus having ascertained the total male births from July 1st 1890 to June 30th, 1900, and subtracting from the result the total number of deaths of males under one year of age in the ten years 1891–1900, we obtain the population out of which the deaths at the age 1—2 occur during the same period. Subtracting the deaths at the age 1—2 we obtain the number out of which the deaths at the age 2—3 occur; subtracting these we obtain the population out of which the deaths at the age 3—4 occur; and subtracting these we obtain the population out of which the deaths 4—5 occur.

The following example will make the above and subsequent steps clear:

First Method:

Male Births July 1, 1890 to June 30, 1900	= 18,490·5
Male Deaths under one year, 1891–1900	= 3,036·0
Population at beginning of second year of life	= 15,454·5
Male Deaths at age 1—2, 1891–1900	= 718·0
Population at beginning of third year of life	= 14,736·5
Male Deaths at age 2—3, 1891–1900	= 307·0
Population at beginning of fourth year of life	= 14,429·5
Male Deaths at age 3—4, 1891–1900	= 185·0
Population at beginning of fifth year of life	= 14,244·5
Male Deaths at age 4—5, 1891–1900	= 122
Population at beginning of sixth year of life	= 14,122·5

Thus the population at birth is 18,490·5
at age 1 is 15,454·5
at age 2 is 14,736·5
at age 3 is 14,429·5
at age 4 is 14,244·5
77,355·5

The mean population for each of these years of life except the first is taken as the geometrical mean of the population at the beginning and at the end of the year, *i.e.* their logs are in arithmetical progression. Thus

$$\log 14{,}244{\cdot}5 = 4{\cdot}1536473$$
$$\log 14{,}122{\cdot}5 = 4{\cdot}1499116$$
$$\text{Diff.} = {\cdot}0037357$$
$$\tfrac{1}{2}\,\text{Diff.} = {\cdot}00186785$$
$$4{\cdot}1499116 + {\cdot}00186785 = 4{\cdot}15177945,$$

therefore mean population during fifth year of life $= 14{,}183{\cdot}4$.

The mean population for the first year of life is obtained by subtracting the deaths under 6 months from the number of births. The results thus obtained are:

Mean population in 1st year of life			$= 16{,}348{\cdot}5$
,,	,,	2nd ,,	$= 15{,}091{\cdot}2$
,,	,,	3rd ,,	$= 14{,}582{\cdot}2$
,,	,,	4th ,,	$= 14{,}336{\cdot}7$
,,	,,	5th ,,	$= 14{,}183{\cdot}4$

Total years of life at risk at ages $0—5 = 74{,}542{\cdot}0 = N$.

But the same total derived from the census returns is 72,067. Hence each of these five mid-year totals must be reduced in the proportion of $\frac{72067}{74542}$ to make them tally with the census returns. The mean populations thus obtained are:

P_0	15,806
P_1	14,590
P_2	14,098
P_3	13,861
P_4	13,712
	72,067

Second Method:

The second and more accurate method takes into account the births in the $4\tfrac{1}{2}$ years preceding the beginning of the decennium. An exact description of this method has been given by Dr Hayward [3], and if the steps of the process are exactly followed no difficulty need arise.

Thus the total population at birth in $1891–1900 = 18{,}490{\cdot}5$.

Population at age 1 equals $\tfrac{1}{2}$ births in 1889 *plus* births in 9 years 1890–98 *plus* $\tfrac{1}{2}$ births in 1899 *minus* deaths under 1 year in 10 years 1890—99

$$= 18{,}430{\cdot}5 - 3039 = 15{,}391{\cdot}5.$$

Population at age 2 equals $\tfrac{1}{2}$ births in 1888 *plus* births in 9 years 1889–97 *plus* $\tfrac{1}{2}$ births in 1898 *minus* the sum of deaths under 1 year in 1889–98 and at age 1—2 in 1890—99

$$= 18{,}320{\cdot}5 - (2944 + 766) = 14{,}610{\cdot}5.$$

Population at age 3 equals $\tfrac{1}{2}$ births in 1887 *plus* births in 1888–96 *plus* $\tfrac{1}{2}$ births in 1898 *minus* the sum of deaths under 1 year in 1888–97, at age 1—2 in 1889–98, and at age 2—3 in 1890-99

$$= 18{,}248{\cdot}5 - (2852 + 801 + 321) = 14{,}274{\cdot}5.$$

Population at age 4 equals $\frac{1}{2}$ births in 1886 *plus* births in 1887-95 *plus* $\frac{1}{2}$ births in 1896 *minus* the sum of deaths under 1 year in 1887-96, of deaths at age 1—2 in 1888-97, at age 2—3 in 1889-98, and at age 3—4 in 1890-99

$$= 18{,}216 - (2850 + 819 + 326 + 187) = 14{,}034.$$

Thus population at birth $= 18{,}490 \cdot 5$
,,   ,,   end of 1st year of life $= 15{,}391 \cdot 5$
,,   ,,   ,,   2nd   ,, $= 14{,}610 \cdot 5$
,,   ,,   ,,   3rd   ,, $= 14{,}274 \cdot 5$
,,   ,,   ,,   4th   ,, $= 14{,}034$

The mean population for each of these years of life is obtained for the first year of life by subtracting the deaths under 6 months of age from the population at birth and for each of the next four years by subtracting half the deaths in the same year of life during the ten years 1891–1900. The mean population 0—1 = 16,348·5, at age 1—2 = 15,032·5, at age 2—3 = 14,457, at age 3—4 = 14,182, at age 4—5 = 13,973. These mean populations are reduced in the manner shewn under the first method, so that their sum may coincide with the total population for the age-group 0—5 ascertained from the census enumerations.

The mean populations of the first five years of life by the two methods are as follows:

Old method	New method
15,802	15,923
14,587	14,641
14,095	14,081
13,858	13,813
13,725	13,609
72,067	72,067

In the present Life Table the figures given by the second method have been adopted. The differences in the expectation of life caused by change from the one method to the other are only changes of distribution between these five years of life, no other years of life being affected.

A reference to Plate XIV. (p. 310) and Fig. 1 (p. 316) shows that although the values of p_0 to p_4 inclusive have been obtained by a different process from that adopted for subsequent ages, the two series are not discontinuous to any material extent. The only point at which "smoothing" would have produced a better curve is p_{5-6} in the male curve. No "smoothing" has been attempted, as it had not been done in the preceding Brighton Life Table.

Years of life at risk at age 85 and upwards.

From age 85 upwards the application of the graphic method becomes difficult and the results unsatisfactory. On reference to the tables on pp. 298, 301 it will be seen that the male years of life at risk at 85

and upwards number only 1055, while the deaths among these are 293. These numbers give curves, the ordinates of which on the scale adopted for the rest of the Life Table cannot be read with correctness. A difference of 5 when dealing with ordinates of the height of 100 assumes an importance which it does not possess when dealing with an ordinate of the height of 10,000. Apart from this mechanical difficulty, which can be met by constructing the curve of this part of the Life Table on a specially large scale, experience shows that the data as to the number of lives at risk and ages at death at these extreme ages are too scanty to suffice for the calculation of life probabilities, and are less in accordance with facts than those at less advanced ages. Consequently whether the graphic or the analytical method of construction is adopted, the data for this part of the Life Table must be abandoned and the foundation figures must be based on theoretical considerations, involving the application of the laws of probabilities. For instance it may be safely assumed that with very few exceptions the whole of the 100,000 (p. 311) started with at birth have died before reaching the 100th year of life. It may also be taken as certain that the probability of living a single year steadily declines after the age of 85.

If the graphic method be followed after the age of 85, it is found that according to the data of the Brighton Life Table the probability of living one year does not follow a regular course, in the case of males when the age 87 has been passed. The female curve also becomes irregular at the same age. It is better therefore to assume for these ages a steadily decreasing value of p_x until all the lives have become extinct. For the sake of exactitude the method of differences has been here adopted.

The logarithms of the p_x values for ages 55, 65, 75, and 85 are differenced, as shewn in the following example :

	1st difference	2nd difference	3rd difference
$\log p_{55} = \bar{1} \cdot 9885151$	$- \cdot 0092959$	$- \cdot 0111731$	$- \cdot 0404793$
$\log p_{65} = \bar{1} \cdot 9792192$	$- \cdot 0204690$	$- \cdot 0516524$	
$\log p_{75} = \bar{1} \cdot 9587502$	$- \cdot 0721214$		
$\log p_{85} = \bar{1} \cdot 8866288$			

In this example the sign of $\log p_{65}$ is first changed and then this log is added to $\log p_{55}$, giving the result $- \cdot 0092959$. This is the first difference of the first term of the series. By similarly treating $\log p_{75}$ and $\log p_{85}$, we obtain the remaining first differences. By treating the first differences in the same way, the second differences are obtained, and so with the third difference.

The last differences opposite each of the series are then added together.   Thus

$$- \cdot 0404793$$
$$- \cdot 0516524$$
$$- \cdot 0721214$$
$$- \cdot 1642531$$

Adding this to the last term of the series

$$\bar{1} \cdot 8866288$$
$$- \cdot 1642531$$

we obtain $\bar{1} \cdot 7223757$

This is the log of $\cdot 52769 = p_{95}$.

The value of p_{95} having been obtained as above, p_{85} and p_{95} are then plotted out on sectional paper, along with the p_x values of a sufficient number of preceding years to ensure a good sweep for the total p_x curve.   The intermediate values of p from 85 to 95 are then read from the curve.   The curve can be continued beyond 95 in a similar manner.   The effect on p_x values produced by adopting the unmodified graphic method and the combination of the method of differences and graphic method for ages over 85 can be seen from the following table :—

Male probabilities of living one year (p_x).

Age	By graphic method	By method of differences
86	·74500	·74500
87	·73654	·71000
88	·75000	·68500
89	·74163	·66500
90	·75700	·64150
91	·76800	·62000
92	·78600	·59600
93	·79200	·57500
94	·70580	·54980
95	·77700	·52770
96	·81800	·50200
97	·79000	·47500
98	·78000	·44800
99	·60000	·42400

Probability of living one year.

The number of years of life at risk at each age and the number of deaths in the corresponding years of life being now known (Tables, pages 298, 301), the probability of living through one year can be stated.

PLATE XIV.

Probability of Living One Year at each Year of Age (curved line); and Average Probability of Living One Year for Age-Groups (horizontal lines).—Males.
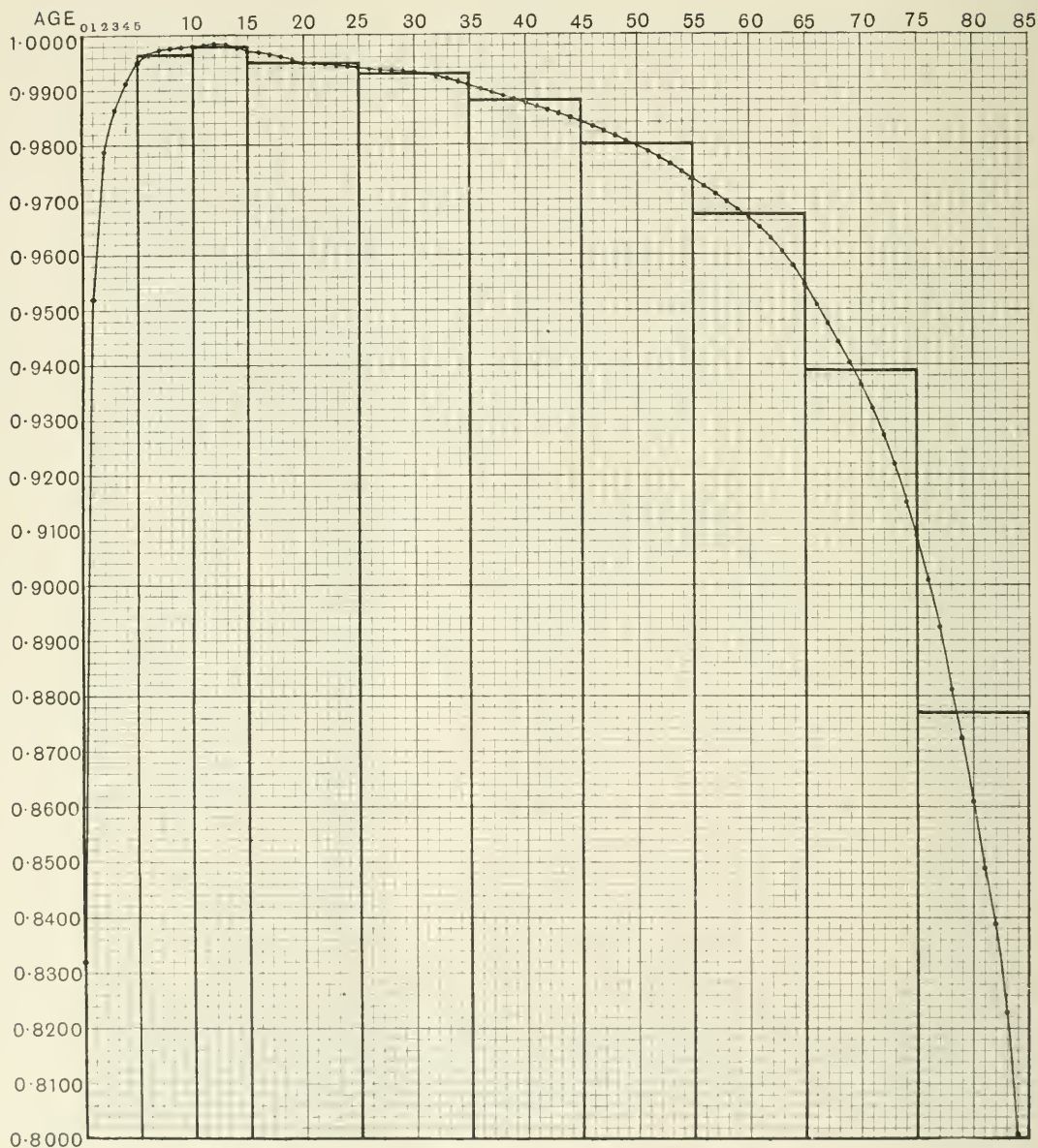
The mean population for each year of life (P_x) has been ascertained from the curve (Plate XII.), and the number of deaths during each year of life (d_x) has been similarly ascertained (Plate XIII.). For every year of life, except the first, it is assumed that the deaths are equally distributed throughout the year. Hence the number living at the end of the year x is equal to $P_x - \frac{1}{2}d_x$, and the number living at the beginning of the year is equal to $P_x + \frac{1}{2}d_x$, and probability of living from the age x to the age $x + 1 = \dfrac{P_x - \frac{1}{2}d_x}{P_x + \frac{1}{2}d_x} = p_x$.

By this means p_x for every year of life except the first can be calculated. Thus—

$$\log p_{5-6} = \log(14,400 - 33{\cdot}5) - \log(14,400 + 33{\cdot}5)$$
$$= 4{\cdot}1573510 - 4{\cdot}1593717$$
$$= \bar{1}{\cdot}9979793$$

therefore $\qquad\qquad p_{5-6} = {\cdot}9953582.$

p_x for 1st Year of Life. The deaths in this year of life are very unequally distributed. Thus in Brighton in 1891–1900 out of 3036 male deaths under 1 year of age, 2142 occurred in the first and 894 in the second half year.

The probability for the first year of life is obtained by dividing the mean population *minus* the deaths during the second six months by the mean population *plus* the deaths during the first six months of life.

Thus $\qquad\qquad p_{0-1} = \dfrac{P_0 - 894}{P_0 + 2142} = {\cdot}8319402.$

Construction of Life Table.

Having obtained p_x for each year of life separately for the two sexes we can now build up the Life Table step by step. It is usual to start with 100,000 children at birth. In Brighton during 1891–1900 the births of male and female children were in the proportion of 50,614 to 49,386, making 100,000 of both sexes. The numbers 50,614 and 49,386 are therefore taken as the number at age 0 in the l_x column of the male and female Life Tables.

Starting with 50,614 male infants at birth, the number living at the end of one year is obtained by multiplying this number by the probability of surviving to the end of the first year.

Thus $50,614 \times {\cdot}8319402 = 42108.$
Similarly $42,108 \times {\cdot}9521333 = 40,092$, and so on.

In order to obtain the mean expectation of life for each individual we must ascertain the total number of years lived by the individuals under consideration, and divide the sum by the number of individuals living this total number of years. The l_x column of the Life Table, a few entries of which are appended, gives the necessary data for this calculation :

Age	Probability of living from age x to age $x+1$ p_x	Born and surviving at each age l_x	Sum of the number living, or years of life lived at each age $x+1$ and upwards, to the last age in the Table Σl_{x+1}	Expectation of Life at each age E^0_x
0	·8319402	50,614	2,248,526	44·92
1	·9521333	42,108	2,206,418	52·90
2	·9784324	40,092	2,166,326	54·53
3	·9866960	39,228	2,127,098	54·75
4	·9910755	38,706	2,088,392	54·46
5	·9953582	38,360	2,050,032	53·94
6	·9963215	38,182	2,011,850	53·19
7	·9968735	38,042	1,973,808	52·38
⋮	⋮	⋮	⋮	⋮
94	·54980	51	56	1·60
95	·52770	28	28	1·50
96	·50220	15	13	1·37
97	·47500	8	5	1·13
98	·44800	4	1	·75
99	·42400	1	0	·50

Thus the 42,108 males surviving at the end of the first year of life out of 50,614 born will have each lived a complete year in the first year, or among them 42,108 years. Similarly 40,092 males will live another complete year each in the second year, or among them a further 40,092 complete years; similarly 39,228 complete years of life will be lived in the third year of life, 38,706 in the fourth year, and so on, until all the males started with become extinct in the 100th year of life.

It is evident therefore that the total number of complete years lived by the 50,614 males started with at birth will be

42,108 + 40,092 + 39,228 +......51 + 28 + 15 + 8 + 4 + 1 = 2,248,526

years. As this number of years is lived by 50,614 males, the number of complete years lived by each male

$$= \frac{2,248,526}{50,614} = 44·42 \text{ years.}$$

This result is known as the *curtate expectation of life.*

We have in the above remarks confined our attention to the

PLATE XV. EXPECTATION OF LIFE.—MALES.

1881–90 +··+··+··+   1891–1900 ·—·—·—·—·
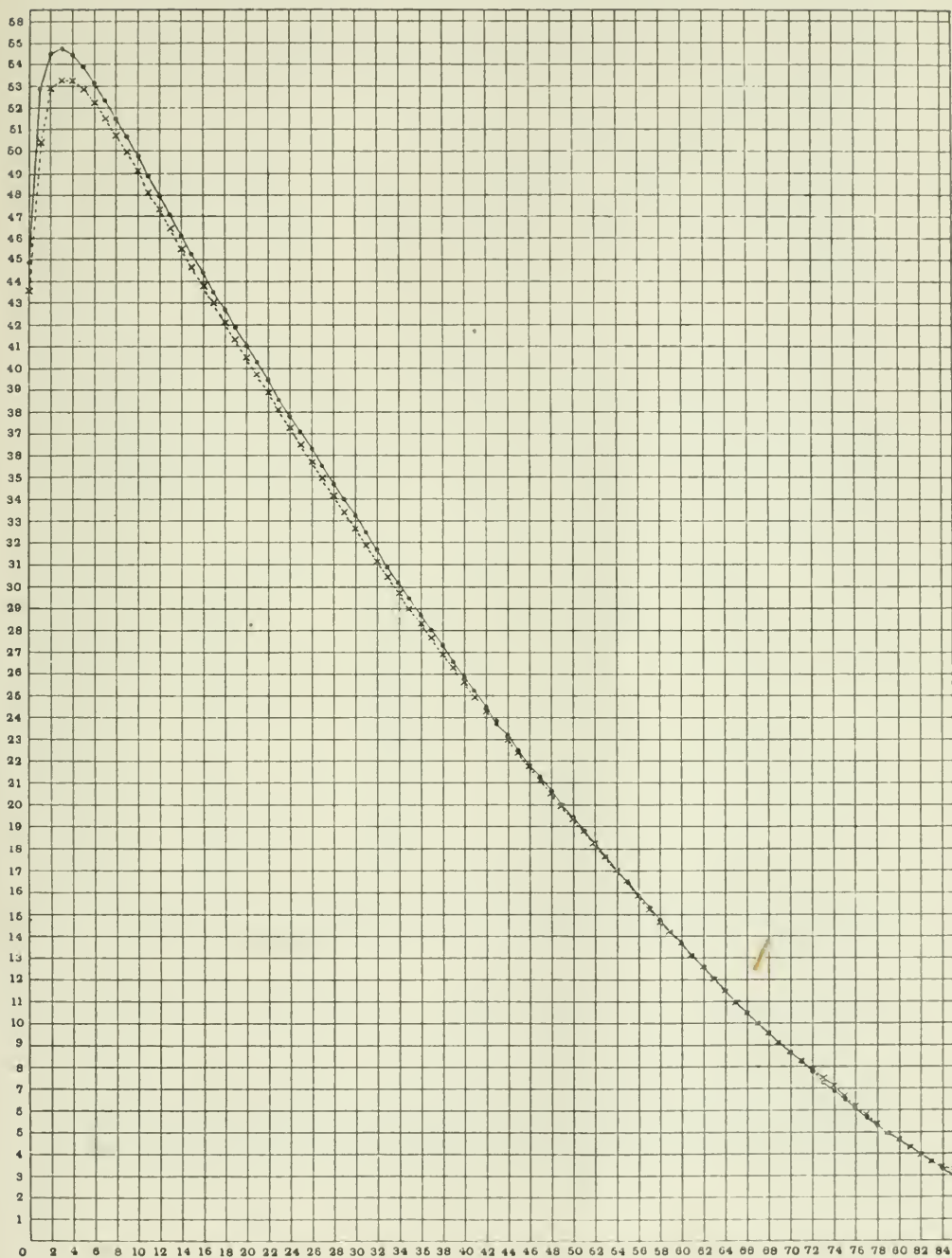
complete years of life, and have not taken into account 'hat portion of lifetime lived by each person in the year of his death. In some instances this may be only a few days, in others nearly an entire year; but it may be assumed with a fair degree of accuracy, taking one person with another, that the duration of life in the year of death will be half-a-year.

If we add this half-year to the curtate expectation of life, the *complete expectation of life* is obtained.

Thus, the complete expectation for males at birth = 44·42 + ·5 = 44·92 years ; at the age of 10 years = 49·30 + ·5 = 49·80 years.

In the preceding table only the complete expectation of life is printed.

A BRIGHTON LIFE TABLE CONSTRUCTED BY THE SHORT METHOD.

In the following table are given the E_x values for certain years of life obtained by the modified short method of constructing a life table, alongside the corresponding values obtained by the preceding graphic method. As this method has been previously described [6], it is unnecessary to give any details as to calculations.

Expectation of Life—Brighton—*Males* 1891—1900.

Age	Graphic method	Short method	Differences
0	44·92	44·89	− ·03
5	53·94	53·89	− ·05
10	49·80	49·75	− ·05
15	45·29	45·24	− ·05
25	37·12	37·06	− ·06
35	29·45	29·40	− ·05
45	22·45	22·50	− ·05
55	16·44	16·41	− ·03
65	11·01	11·01	*nil*
75	6·49	6·60	+ ·11

A similar closeness has been observed between the results obtained by the short method and by Hayward's extended analytical method [8].

The Graphic and the Analytical Methods of Life Table Construction.

As already indicated, neither the graphic nor the analytical method of constructing a Life Table can be relied upon to give accurate results for ages 0–5, nor, when based on the data for the years of life in question, for ages 85 and upwards, owing to the untrustworthiness

at these ages of the census and death returns. Hence at the extremes
of life special processes require to be adopted (pages 304 and 307).
For intermediate ages the question arises whether the graphic or
the analytical method is preferable.

1. *Difficulty of application and special knowledge required.* For
many Medical Officers of Health the answer to the above question
will be determined by the decision as to which method is the easier
and more expeditious, unless it can be shewn that the easier process
is markedly less accurate. On these grounds the graphic is undoubtedly
preferable and should in our opinion be chosen. (By the phrase
"analytical method" is meant the extended analytical method unless
otherwise stated.) The above remark does not apply to the Short
Method of Farr, as modified and improved by Dr Hayward. Nor
does it apply to the combined graphic and analytical method
described by Dr Hayward [9]. Both these methods involve less labour
than the extended graphic method described in this paper. We
therefore propose to compare certain results obtained by these three
methods.

(*a*) This is done for the Modified Short Method on page 314.
It will be noted that if the age 75 be excluded from consideration the
differences in the expectation of life at quinquennial intervals of age
never exceed ·06 part of a year, when the Modified Short Method
is compared with the Graphic Method. The greater difference at age
75 is relatively unimportant, as expectations of life at this age are
necessarily based on somewhat uncertain data.

(*b*) In Fig. 1 A and B the p_x values obtained by the graphic method
for ages 5 to 15 are compared with the corresponding values obtained
by the combined graphic and analytical method (grade 1)[1]. This latter
method is based on and has been shown by Dr Hayward to give results
closely approximating to those given by his improved extended analytical
method. The ages 5–15 are the period of life at which the p_x values
obtained by different applications of the analytical method have in actual
experience shown the greatest discrepancies. It will be seen that the
correspondence between the two curves is extremely close, as may
be seen also by reference to the following table. The results obtained
by the graphic and by the combined method are for the most part

---

[1] Strictly speaking the $\log p_x$ values should have been plotted out in Fig. 1. This
was done and the results coincided with those shown in Fig. 1. The p_x values have
been plotted out in this figure by preference, because the p_x curve brings out more clearly
any differences between the results obtained by the two methods.
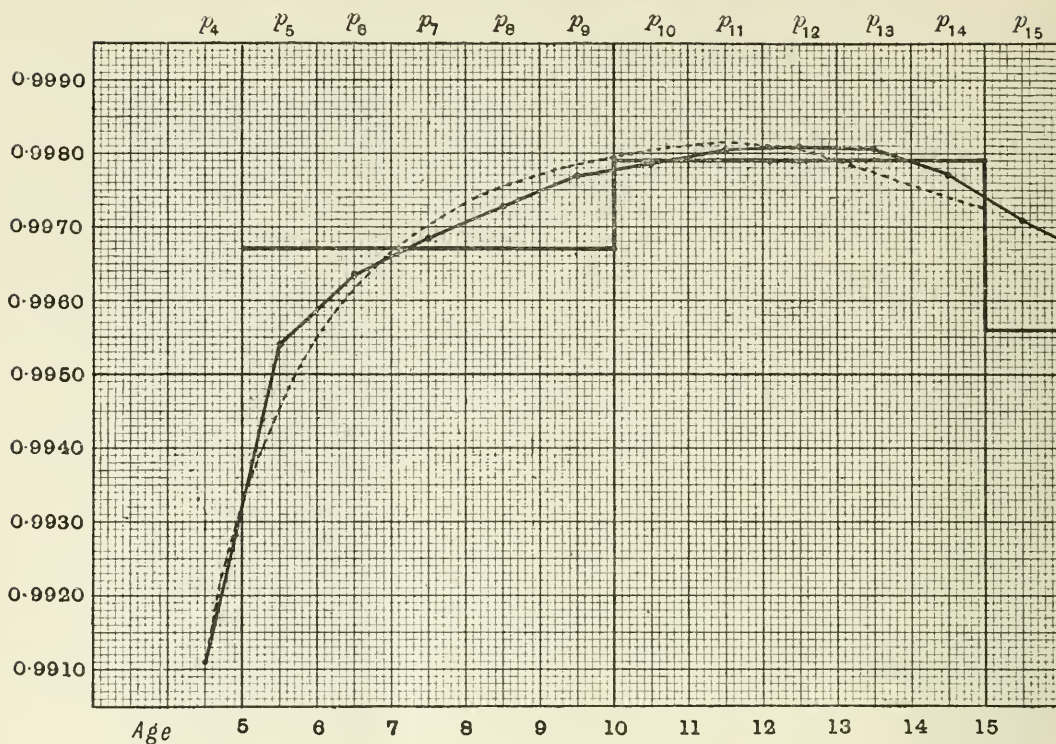
Fig. 1. A.
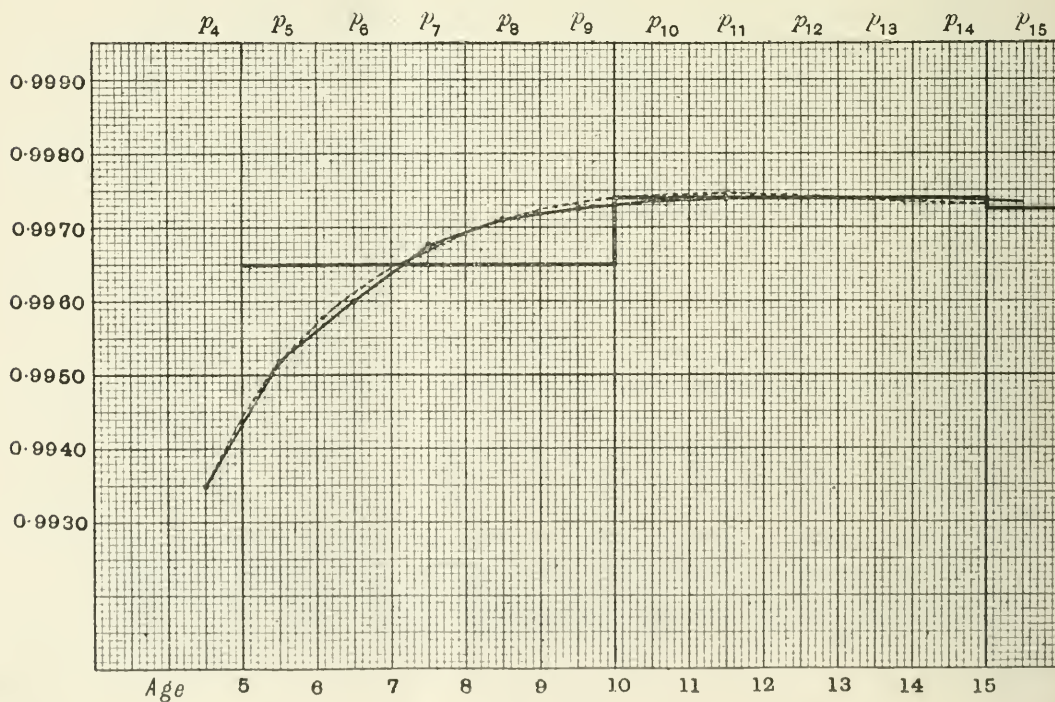
Fig. 1. B.

Fig. 1. Comparison of p_x values by Graphic Method and by Combined Method for ages 5—15.

A. Males.   B. Females.

practically identical; a fact which strongly supports the value of the empirical rules for drawing the p_x curve for ages 5–15, and indirectly strongly confirms the accuracy of the extended analytical method which Dr Hayward after repeated trials has adopted.

Table comparing Probabilities of Life by the Graphic and Combined Methods.

p_x *values.*

Age	Males			Females		
	Combined Method	Graphic Method	Difference	Combined Method	Graphic Method	Difference
5	·99456	·99536	+ ·00080	·99518	·99519	+ ·00001
6	·99616	·99632	+ ·00016	·99614	·99600	− ·00014
7	·99708	·99687	− ·00021	·99671	·99675	+ ·00006
8	·99761	·99729	− ·00032	·99710	·99710	± ·00000
9	·99786	·99770	− ·00016	·99733	·99725	− ·00008
10	·99804	·99785	− ·00019	·99740	·99734	− ·00006
11	·99814	·99805	− ·00009	·99742	·99742	± ·00000
12	·99805	·99807	− ·00002	·99740	·99743	− ·00003
13	·99777	·99805	− ·00028	·99740	·99741	+ ·00001
14	·99740	·99766	− ·00026	·99740	·99739	− ·00001

From the facts already given it is clear that so far as Brighton is concerned the short method, the graphic method, and the combined method all give approximately identical results. Similarly for England and Wales Dr Hayward has obtained approximately identical results by the use of the short method and of his improved extended analytical method. The conclusion is inevitable that the results obtained by these four methods are practically correct, and that the choice as to which method shall be adopted is one to be determined largely by considerations of convenience and personal preference.

We have preferred the detailed graphic method, by which we have obtained results strictly comparable with those of 1881–90; but our experiments with the other two methods, which are decidedly less laborious, indicate that the differences of values of E_x are so small that one of the other methods might have been adopted without destroying the comparability of the 1891–1900 Brighton Life Table with that of 1881–90, or with other Life Tables constructed by the most modern analytical methods.

In the remarks which follow it must still be noted that we are comparing the graphic method with the extended analytical methods.

2. *Accuracy of Methods of Determination of Facts for Each Age.* In insurance experience the number of lives at risk and the number dying at each age are exactly known, but in national and district Life

Tables we are concerned with census returns and death returns, in both of which ages are often erroneously stated, with a special run on round numbers such as 10, 20, 30, etc.   In passing it may be noted however that actuaries, who have more accurate data than those provided by the census and death returns of the whole country or of a particular town, appear generally to prefer the graphic method of applying their data. In local and national returns of population and deaths, the data are only available in age periods 0–5, 5–10......25–35, 35–45, etc., or where given for single years of life cannot be trusted.   It is necessary therefore to adopt some method of interpolation for single years of life, and this can be done by graphic or analytical processes.   In the analytical method this is done by the method of interpolation by finite differences.   Thus if the age 35 is being dealt with the facts at ages 15, 25, 45, and 55, are taken into account if four orders of differences are being used.   In the graphic method these ages are also taken into account.   There is "only one curve for all the parallelograms" (Plate XII.) " not a curve for each[1]."   Although in each parallelogram the accuracy of the curve is determined by the fact that the sum of the ordinates equals the area of the corresponding parallelogram, this equality might be secured by many other curves for the years of life in question than the one actually constructed.   The possibility of this variation is almost without exception prevented by the fact that the part of the curve relating to the parallelogram in question must fit into the general sweep of the curve for neighbouring parallelograms.   Hence in practice it is found that one curve and one curve only can as a rule be constructed in which (1) the sum of the ordinates shall equal the area of the parallelogram, and (2) the sweep of this part of the curve shall fit in with the general sweep of the neighbouring parts of the curve.   The only exception is when the curve changes from an upward to a downward direction or *vice versa* (see footnote, p. 304).   The accuracy of these results is confirmed by the facts that the p_x curve (Plate XIV.) and the E_x curve (Plate XV.) based on the original curves of population and deaths run smoothly, although with the exception referred to above there has been no "smoothing," the curves exactly representing the calculated results.   This meets the main objection urged against the graphic method, that the curves will vary with the individuality of the draughtsman.   The objection that the heights of ordinates are difficult to measure is more theoretical than practical, as the possible differences

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[1] King, *op. cit.*

of reading throughout the greater part of life when an adequate scale is employed have no appreciable effect upon the E_x values. Near the end of life the error is obviated by drawing the curve on a larger scale.

3. *Diversity of Analytical Methods.* From the above facts it is clear that except at one or two points it is within narrow limits only practicable to secure one graphic curve interpreting the data. Can the same be said for analytical methods? In practice most discrepant and irreconcilable results are obtained when analytical methods are employed, owing to the fact that no one system of interpolation has been uniformly adopted. Two examples of this fact may be adduced.

(1) Dr Hayward on applying his new method of interpolation to the data of the Manchester Life Table for 1881–90 obtained the following results:

	New values	Values in the published Life Table	Differences of new from published values
E_0	35·10	34·71	+0·39
E_5	46·16	45·59	+0·41
E_{10}	42·86	42·75	+0·11
E_{15}	38·62	38·78	−0·16
E_{20}	34·63	34·62	+0·11

The considerable divergencies at the above ages depend upon different modes of interpolating the p_x values from age 5 to age 25. The corresponding discrepancy for p_x values is shown in Fig. 2, where the dotted line shows the values in the published Life Table, and the continuous line the values in the Life Table calculated by Dr Hayward.
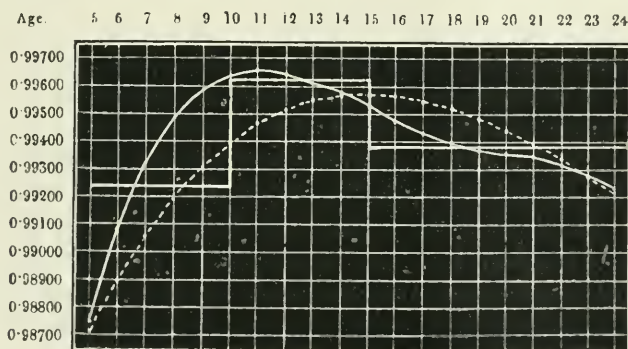
Fig. 2.

(2)   A further instance may be quoted from Dr Hayward's paper[10]. In his diagram here reproduced (Fig. 3) the continuous line gives the p_x values for ages 5 to 24, as calculated by him, while the two dotted curves give p_x values derived from the same data by two other methods which have been employed in Life-Table construction. To take the instance of one year, $p_{10} = \cdot 9940$ by one method, and $\cdot 9984$ by another.
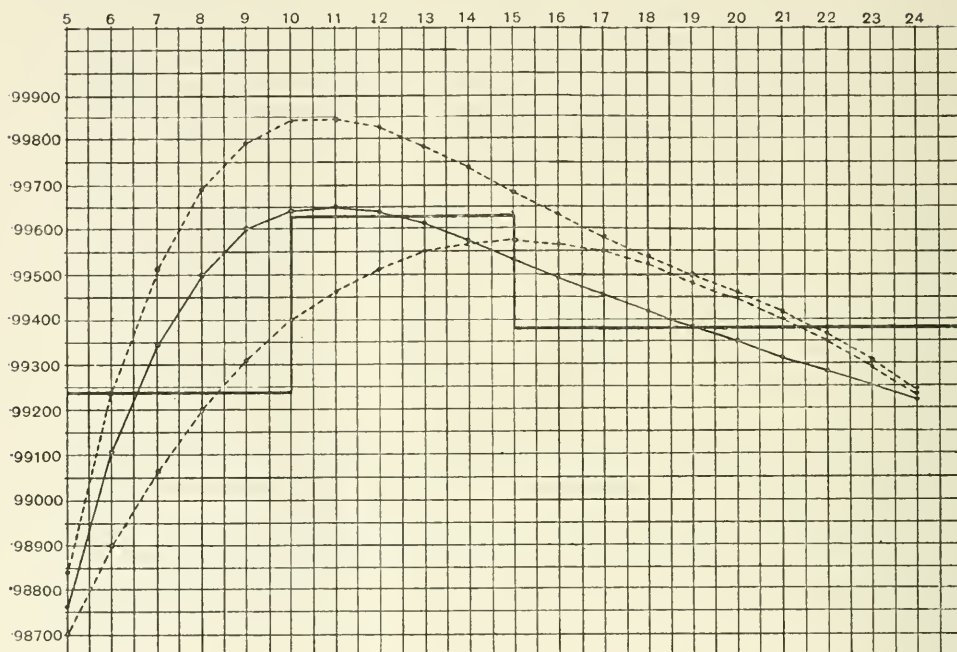
Fig. 3.

In contrast with the above examples of discrepant results obtained by the use of different analytical methods, the closeness of the correspondence between results obtained by the graphic method and by the most modern and accurate analytical methods may be adduced. We refer to the methods described by Dr Hayward[11] and employed by Mr Shirley Murphy[12] in the London Life Table for 1891–1900, which in the case of Brighton have been shown (p. 316, Fig. 1) to give results closely corresponding with those obtainable by the graphic method.

A further instance of the same close correspondence between results obtained in the Brighton Life Table constructed ten years ago by the graphic method, and corresponding results obtained by an extended and

improved analytical method, is shown in the following table, in which the values obtained by the analytical method have been calculated by Dr Hayward.

Brighton (Males) Life Table, 1881–90.

	Graphic Method ("G")(Newsholme)	Analytical Method ("A")(Hayward)	Differences of "A" from "G"
E_0	43·59	43·56	− ·03
E_5	52·87	52·88	+ ·01
E_{10}	49·12	49·12	± ·00
E_{15}	44·67	44·66	− ·01
E_{20}	40·55	40·51	− ·04
E_{25}	36·51	36·51	± ·00
E_{35}	29·02	29·04	+ ·02
E_{45}	22·36	22·36	± ·00
E_{55}	16·48	16·46	− ·02
E_{65}	10·96	10·94	− ·02
E_{75}	6·64	6·58	− ·06
E_{85}	3·33	3·46	+ ·13
E_{95}	1·68	1·22	− ·46

The discrepancies are insignificant until after the age of 75, after which age they are unimportant.

4. *The analytical appeals to the graphic method in selecting the best method of interpolation.* With analytic methods of Life Table construction an indefinite number of applications of the process of analysis are available, and the various applications employed have been shewn above to give discrepant results, the differences sometimes amounting to as much as a year in the case of a particular E_x value. The particular mathematical process to be adopted is therefore "by no means a matter of indifference," and no guidance appears to be furnished by mathematics as to the special method to be adopted. Thus we find Dr Hayward, one of the ablest exponents of the analytical method, appealing in this embarrassment of choice, not to mathematics, but to the graphic method. Referring to the instance of Manchester already given, he constructed [11] eight different p_x curves for the years of life 5—15, by eight different applications of the method of interpolation, and based his choice from amongst these solely upon graphic considerations. In other words, he constructed an "ideal curve" representing the known facts as closely as possible, and then by a process of elimination selected the curve obtained by analytical methods which differed least from it. The fact that by this means he was able to arrive at an analytical method which as tested by our application of the graphic method gives correct results, does not appear

to us to detract from the force of the contention that analytical methods are inconclusive and give no guidance as to which analytical process gives results most in accordance with the facts. It appears preferable, since the " ideal curve," *i.e.* the curve nearest the known facts, forms the ultimate appeal to which the selection of analytical methods must be referred, to adopt the " ideal curve " itself rather than any approximation, however close, *i.e.*, to adopt the graphic method. The authors of other analytical Life Tables so far as we are aware have furnished no information as to the considerations guiding their choice of method; but since in the London Life Table for 1891—1900 the same series of u_x values has been adopted from which to calculate the critical p_x values [1] for ages 5—15 as that selected in the manner above described, the London Life Table may also be regarded as constructed by the application of methods based on graphic considerations.

From the preceding facts it is clearly unsafe to compare Life Tables constructed by different analytical methods. By so doing most erroneous deductions may be obtained. In the National Life Table for 1881—90 an improved method of construction was employed. In the Decennial Supplement of the Registrar-General for 1881—90 [13] it is stated " for ages 45 and upwards the expectations of life are lower by the new table than by either of the others " (*i.e.*, for 1834—54 and for 1871—80). The following table shows in the first two columns the results indicated in the above quotation; and in the next two columns the results obtained by Dr Hayward [14] when the two Life Tables are constructed by a uniform method.

Expectations of Life.

Age	By Official Tables		Recalculated by a uniform method		Difference of B from A	Difference of b from a
	A 1871—80	B 1881—90	(*a*) 1871—80	(*b*) 1881—90		
35	28·64	28·91	28·40	28·87	+ ·27	+ ·17
45	22·07	22·06	21·88	22·04	− ·01	+ ·16
55	15·95	15·74	15·66	15·71	− ·21	+ ·05
65	10·55	10·31	10·21	10·24	− ·24	+ ·03
75	6·34	6·10	5·91	6·06	− ·24	+ ·15
85	3·56	3·29	3·15	3·32	− ·27	+ ·17
95	2·01	1·72	1·57	1·72	− ·29	+ ·15

[1] $u_x = 2P - d$ at ages x and upwards.

It will be seen that the expectation of life at ages 45 and upwards has not declined but actually increased. Hence until the authors of these tables agree upon the adoption of some one scheme of construction to be strictly followed in all instances, the full value of their work will not be secured, since one of the main functions of Life Tables is to enable accurate comparisons to be instituted for each sex between the vital conditions of populations differing from each other in geographical distribution, time, social status, sanitary condition, etc.

Summary as to Graphic and Analytical Methods.

(1) The graphic method is more easy of application and requires less mathematical knowledge than the extended analytical method.

(2) In our experience it produces smooth curves of p_x and E_x.

(3) The analytical in the selection of the best process of interpolation appeals to the graphic method.

(4) While either the analytical or graphic process may in unskilful or careless hands give erroneous results, owing to errors in working out, the analytical process, unlike the graphic, presents a wide choice of methods, which although accurately worked out, give incomparable results.

(5) The facts given in the table on page 321 and in Fig. 1 A and B and table on page 317 show that almost identical results are obtained by the graphic method and by the improved extended analytical method. We regard this as important testimony to the accuracy of each of these methods, and at the same time as indicating the inaccuracy of the analytical methods giving different results.

(6) As the results obtained by the modified short analytical method and by the combined analytical and graphic method approximate so closely to those obtained by the detailed graphic method, either of them may safely be adopted by those who cannot spare time for working out a detailed Life Table by the graphic method.

(7) Every published Life Table should give exact details of the method of its construction, in order that the comparability of data and results may be tested. Much confusion has arisen, and important errors in comparison between different Life Tables have been produced, by the use of different methods of construction and the non-publication of details of methods.

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(6) Hayward. *Journal of Hygiene*, Vol. II. No. 1, p. 12.

(7) Do. page 34.

(8) Do. page 40.

(9) Do. *Journal of Hygiene*, Vol. II. No. 2, p. 207.

(10) Do. *Journal of Hygiene*, Vol. II. No. 1, page 28.

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(12) The London Life Table (Based on the Mortality in the Decennium 1891—1900). Shirley F. Murphy.

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THE FACTORS WHICH DETERMINE THE LOCAL INCIDENCE OF FATAL INFANTILE DIARRHOEA.

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THE various problems of infantile mortality are not only supremely interesting to the medical officer of health, but, with the continued decline in the birth rate, are calculated to assume even greater practical importance in the near future. Of these problems none is more in need of solution than the exact etiology of infantile diarrhoea.

It is moreover one which the writer has repeatedly had to investigate during the last seven years under such diverse conditions as obtain in a small manufacturing town in North Derbyshire and in a large residential borough in Surrey.

In Chesterfield, during the years 1896 to 1899, the infantile death rate amounted to 195 per 1,000 births, while the deaths under one year from diarrhoeal diseases[1] equalled 54·4 per 1,000 births, ranging from 44 in 1896 to 83·1 in 1897.

In Croydon on the other hand the infantile mortality rate is considerably lower, but there are and have been indications that this rate is hardly as satisfactory as might have been expected in such an exceptionally healthy town. Indeed while the general death rate maintains its low level there appears to be a tendency for the infantile mortality figures to approximate more and more closely to those for London and for England and Wales.

For instance an examination of the figures for the last twenty-two years gives the following results:

TABLE I.

Years	Croydon	London	England and Wales
1881—1890	120	152	142
1891—1900	139	160	154
1901	140	149	151
1902	133	141	133

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[1] Throughout this paper the term "diarrhoeal diseases" will include deaths from diarrhoea, zymotic enteritis, gastro-enteritis, and enteritis, *i.e.* schedules 10, 11, and 107 of the Society of Medical Officers of Health.

While my predecessor's reports for the years 1883 to 1899 and my own statistics for the years 1900 to 1902 suggest that, as far as the assigned causes of death are to be relied on, diarrhoeal diseases bulk more largely than formerly in the mortality tables.

Table II.

Infantile Mortality in Croydon during the last twenty years.

	Total Infantile Mortality per 1000 Births	Deaths under 1 from Diarrhoea and Enteritis per 1000 Births	Infantile Mortality from other causes than Diarrhoea
1883—1887	118	16	102
1888—1892	116	10	106
1893—1897	142	25	117
1898—1902	143	38	105

Before, however, suggesting remedial measures in Croydon, it was decided to collect as much information as possible as to the local distribution and ascertained antecedents of all cases of fatal diarrhoea in infants under one year of age.

The local facts are based on a careful examination of all the deaths under one year in Croydon during the ten years 1893 to 1902, and on special enquiries into deaths from diarrhoeal diseases in Croydon during 1900 to 1902, amplified by similar information collected in Chesterfield during the years 1896 to 1899.

In order to measure the local incidence with exactness, attention has been confined (unless otherwise stated) to deaths under one year, as these can be accurately measured in terms of the total annual or decennial births. In the following pages an attempt has been made to apply these facts to current etiological hypotheses, with a view of ascertaining how far they could account for the local phenomena. The results may be of some general interest because, as will be shown later on, Croydon is a borough with a comparatively large acreage (9012 acres) divided into sub-districts which differ widely in the geological character of the soil, in the physiographical conditions of the surface, and in the social status of the inhabitants.

What is known as to Etiology of Diarrhoea?

Physicians, bacteriologists, and epidemiologists have all made valuable and lengthy contributions to the subject. Their most important suggestions will be considered in detail later; but briefly stated, physicians are agreed that fatal diarrhoea affects infants brought up by

hand rather than those which are breast-fed, and attribute the bulk of it to want of care or knowledge in the preparation of infants' food.

Bacteriologists on the other hand do not for the most part speak with any certain voice. *Streptococci, Bacillus sporogenes enteritidis, Bacillus proteus* have all been described[1] as causes of epidemic diarrhoea. More recently Prof. Delépine[2] has brought forward further reasons for suspecting members of the coli group as the most common cause of summer diarrhoea.

Epidemiologists appear to have proved that diarrhoea mortality is most frequent among the poor; is associated with urban conditions; is mainly confined to the third quarter of the year; is more common in hot, dry summers than when those periods of the year are cool and wet; is probably more common where the soil is polluted with organic matter and where the physical conditions (such as permeability and surface slope) do not lend themselves to natural cleansing by showers of rain.

Yet in spite of all this accumulated labour it cannot be said that our knowledge is sufficiently precise to afford sure guidance in the selection of remedial measures.

Need for detailed information as to antecedent processes.

In a recent address[3] Prof. Allbutt declared that he was sick of diseases and wanted to " know processes and origins." Those who are engaged in the practice of hygiene must often have re-echoed this wish; for it is to the fuller understanding of processes that we must look for guidance in formulating preventive measures. " Processes " indeed are frequently of far greater importance than even "origins," as has recently been demonstrated in the case of malaria and yellow fever. For there can be no doubt that it is the recognition of the part played by mosquitoes in these diseases that is destined to produce practical results. Similarly the discovery of the particular germ or germs concerned in the production of diarrhoea is hardly likely to be fruitful unless the precise avenues of infection are clearly demonstrated.

" Diarrhoea," it must also be remembered, is simply the name of an obvious symptom which may result from such diverse causes as a purgative, nervous shock, mechanical irritation, or from some general infection. Even the symptom-complex described by Ballard[4] in his

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[1] Gordon, *Practitioner*, August 1902.
[2] This *Journal*, 1903, Vol. iii. p. 68.
[3] *Lancet*, 1903, i., p. 645.
[4] L. G. B. Supplement 1889.

classical report on diarrhoea does not necessarily predicate etiological identity even in cases which appear to the clinician to be precisely similar; and summer diarrhoea, even if proved to be an infective disease, may, however uniform its other antecedents, be as diverse in its bacteriology as ulcerative endocarditis or other forms of pyaemia which result from the invasion of the body by various pyogenic organisms. In any case it seems not unlikely that, as in the case of typhoid fever and polluted water, useful results may be arrived at by purely epidemiological and clinical methods, and that premature dependence on bacteriological inferences may delay the advent of those approximations to the truth which we are all anxious to reach.

It must also be understood that, though the observations are discussed under headings, the various suggested causes are not mutually exclusive, and that though each fatal case of diarrhoea has its own special antecedents there is no reason why the antecedents should be the same in each instance.

What is meant by Diarrhoeal Diseases?

At the onset one is met with difficulties of nomenclature and classification.

Should we adopt the classification of the Registrar-General and exclude from consideration all deaths ascribed to enteritis and gastro-enteritis (*i.e.* Schedule 107 of the Society of Medical Officers of Health)? After careful consideration it seemed unwise to do so. It is true the term "enteritis" probably includes a certain number of deaths that differ in their antecedents from the acute form of diarrhoea which is variously known as "summer diarrhoea," "zymotic enteritis," or "epidemic diarrhoea." Yet there can be no doubt that the majority of cases of "enteritis" differ neither in causation nor in symptoms from the other group. Both "diarrhoea" and "enteritis" are summer diseases, as may be seen from Charts I. and II., and from Table III., and the balance of evidence is in favour of treating them in the aggregate and so avoiding the difficulty that might arise from variations which make it so difficult to institute comparisons between different towns and districts.

The following is a summary of the deaths under 1 year from diarrhoea and enteritis in Croydon during the last decennium.

Table III.

Year	Number of Births	Deaths under 1 year from Diarrhoea and Enteritis	Deaths during 3rd quarter from Diarrhoea and Enteritis
1893	2852	82	65
1894	2805	20	15
1895	2906	77	59
1896	2964	65	54
1897	3034	113	102
1898	3150	161	141
1899	3204	153	134
1900	3270	94	70
1901	3578	137	123
1902	3576	72	56
Total for 10 years	31,339	974	819

Average Diarrhoeal Rate 31·1 per 1000 births.

,, ,, 26·1 ,, ,, in 3rd quarter.

Method of Feeding.

During the last three years enquiries have been made into all deaths under one year of age. In many instances full information could not be elicited; but in 1,008 cases the replies obtained by the Health Visitors were sufficiently full to admit of tabulation. In considering the relation of various methods of feeding to diarrhoeal diseases, all deaths under one week were omitted as they would obviously be of little value. 253 infants aged one week to one year dying from diarrhoea or enteritis were found to have been fed in the following way, and for the sake of comparison are placed in parallel columns with 313 deaths from respiratory and other diseases not likely to be associated with food infection.

Table IV.

	Respiratory Dis., Overlain, Measles, Whooping Cough, "Other" Diseases		Diarrhoea and Enteritis	
Number investigated	313		253	
Age in months	0—6	6—12	0—6	6—12
Breast alone	55 p.c.	43 p.c.	14 p.c.	11 p.c.
Breast and food	1 ,,	1 ,,	1 ,,	2 ,,
Breast and cows' milk	3 ,,	—	1	
Condensed milk	17 ,,	21 ,,	33 ,,	29 ,,
Other prepared foods	1 ,,	3 ,,	3 ,,	4 ,,
Cows' milk	23 ,,	32 ,,	48 ,,	54 ,,

The results are sufficiently striking, especially when one confines one's attention to the proportion of infants fed by the breast alone in

the two classes. It is quite true that the mere fact that only 14 and 11 per cent. of infants dying from diarrhoeal diseases were breast-fed does not prove that diarrhoea results from food infection, as these may be the average methods of feeding infants in the district. In default of accurate figures as to the method employed in the case of surviving infants in Croydon comparison may not unfairly be made with the replies received in the case of respiratory and other diseases not likely, on *a priori* grounds, to be associated with errors in diet.

If we assume that the method of feeding in these cases is the average—then out of 10,000 infants:

5500	are breast-fed alone	during the first six months			
100	have breast and food	,,	,,	,,	
300	,, breast and cows' milk	,,	,,	,,	
1700	,, condensed milk	,,	,,	,,	
100	,, other prepared foods	,,	,,	,,	
2300	,, cows' milk	,,	,,	,,	

Now during the same period there were 191 deaths under six months from diarrhoea and enteritis among 10,424 births or 183 for 10,000 births.

And from the third column of Table IV. it will be seen that these 183 deaths from diarrhoea under six months were distributed as follows :

14 per cent.	or 26	were entirely breast-fed
33 ,,	or 60	were fed on condensed milk
48 ,,	or 88	were fed on cows' milk

During the first six months of life the death rate from diarrhoea was therefore in round numbers

5 per 1000	among the entirely breast-fed	
35 ,,	in those fed on condensed milk	
38 ,,	in those fed on cows' milk	

As a further proof of the relation of methods of feeding to diarrhoeal diseases, the following figures may be quoted. In 1898, 372 deaths of infants in Chesterfield were investigated, and similar enquiries were made in the case of 408 surviving infants with the following results, as regards infants under six months, expressed in percentages.

	Survivors per cent.	Deaths from Diarrhoea per cent.
Breast-fed only	42	11
Breast and food	42	22
Milk and foods	16	67

In other words, while only 16 per cent. of the surviving children were entirely subjected to the risks of hand-feeding, no less than 67 per cent. of those dying from diarrhoeal diseases were so subjected.

These figures are amply confirmed by those of other observers. Those given by Dr Newsholme (*Trans. Epidemiol. Society*, Vol. XXI.) are particularly corroborative. In 191 cases of fatal diarrhoea in Brighton it was found that the method of feeding was as follows:

Breast	9·4 per cent.
Condensed milk	44 ,, ,,

From similar enquiries, Dr Hope finds that if 1000 breast-fed infants under 3 months of age have to encounter the risks of summer diarrhoea in Liverpool, then 20 will succumb. In the case of 1000 hand-fed children 300 would die from that cause. (*Ann. Rep.* Liverpool, 1900.)

There can therefore be no reasonable doubt that hand-feeding is a common antecedent of fatal diarrhoea in infants, and that those who are hand-fed are much more likely to suffer from diarrhoea than are those who receive the food which nature intended for them.

How does hand-feeding tend to produce fatal diarrhoea?

It is necessary however to pursue the enquiry a step further and ascertain the exact processes which tend to make hand-feeding so hazardous. First it might be suggested that hand-fed infants are more weakly than those brought up on the breast and therefore more prone to fall victims to any debilitating complaint. But though breast-feeding is always desirable in the interests of the infant, there can be no doubt that hand-feeding may be perfectly successful if judiciously supervised, and, as Ballard pointed out (*loc. cit.* p. 45), summer diarrhoea does not make its first fatal swoop upon the weakliest children nor confine its attention to the delicate.

Next it might be argued that the poison of diarrhoea is introduced into the homes of the poor by means of milk, and when one considers how very unsatisfactory are the methods of the average dairyman there is much to be said for this hypothesis as has so ably been urged by Prof. Delépine (*loc. cit.*). According to this writer milk which is contaminated with faecal matter at the farm develops toxicity from being kept too long in hot weather. Summer diarrhoea he suggests is strictly analogous to the ordinary forms of food poisoning which have been shown by various bacteriologists to be due to infection with

Gärtner's bacillus or allied members of the coli group. This view is supported by the extremely interesting and valuable observations made on samples of milk collected in various parts of the country during the last ten years and examined by subcutaneous injection into guinea-pigs. It need hardly be pointed out that this hypothesis has the merit of simplicity and, if adopted, would make the selection and direction of preventive measures a comparatively easy matter. All would be relieved to find that diarrhoea was dependent on the intestines of the cow and not on some mysterious change in the bowels of the earth. For, if that were so, adequate control of the milk supply would solve the chief difficulty.

But does Professor Delépine's hypothesis explain the facts? In the first place is diarrhoea really analogous to such epidemics of food poisoning as that which occurred at Derby in September 1902? Now from an examination of the Local Government Board reports and the recent medical journals it would appear that the seasonal incidence is very different. For excluding cases due to tinned food I have been able to find records of some 22 cases of food-poisoning. These occurred at the following seasons:

| First quarter | 5 | Third quarter | 6 |
| Second ,, | 6 | Fourth ,, | 5 |

They therefore differ in the seasonal incidence from fatal diarrhoea, which is practically confined to the third quarter of the year, as is well illustrated in the accompanying charts. Nor do diarrhoea deaths ordinarily occur to any great extent in groups such as those which mark other food epidemics. The interesting epidemic referred to by Professor Delépine (*loc. cit.*, p. 74) is entirely exceptional. Indeed it is because it is exceptional both in season and in grouping that it stands out so clearly from what one sees every summer during the diarrhoea season. Nor must the experiments with guinea-pigs be accepted for more than they are worth. If we are intended to infer that, because 61 per cent. of the milk reaching Manchester from a distance is toxic to guinea-pigs, it is therefore toxic to infants in the sense that the Derby pork-pies were to adults; then it can only be suggested that diarrhoea ought to be even more general and fatal than it is at present. If on the other hand we are to assume that the milk proved toxic to guinea-pigs simply because ordinary faecal contamination was rendered active by the milk being kept too long in hot weather; then the incidence of

summer diarrhoea should vary with the temperature of the air and with the distance that milk has to travel before reaching the consumers.

With regard to the temperature of the air Ballard pointed out that "the influence thus exerted *is not a direct influence,* except in so far as it affects also infant mortality from all causes;" whereas the figures quoted by Prof. Delépine in Tables IV. and V. (*loc. cit.* p. 83) show that the mean temperature of the air had a very direct effect on milk judged by its action on guinea-pigs.

The curves for Croydon however do not show that diarrhoea fatality varies with the temperature of the air. It will be seen, for instance, that in 1902 the highest mean temperature of the air was reached in the 26th week, while there was no prevalence of fatal diarrhoea until the 33rd week. In 1901, again, diarrhoea became epidemic as the mean temperature was falling.

In 1898 the temperature-curve corresponded fairly well with the diarrhoea fatality; but 1898 was an exceptionally dry summer. It should also be noted that diarrhoea deaths did not occur until some weeks after the mean temperature had reached 55° Fahr., the point at which marked toxic effects should be produced by milk coming from a distance according to Prof. Delépine's experiments. In 1894 diarrhoea was practically absent though the mean temperature was above 55° Fahr. for fourteen weeks.

Similarly with regard to the time factor, it is a matter of common knowledge that London does not suffer to any extreme degree from diarrhoea mortality though there is probably no town in the country which draws its milk from a greater distance.

Nor did an analysis of the method of feeding of the fatal cases of diarrhoea lend the least support to the suggestion that such diarrhoea was consequent on the ingestion of milk which came from a great distance. For an examination of Tables III. and IV. (p. 329), shows that a not inconsiderable number of infants dying from diarrhoea and enteritis were breast-fed, while only a little more than half were receiving fresh cows' milk. Nor would it seem probable that condensed milk is primarily infective. In none of the eight samples examined by Dr Klein for the Local Government Board could pathogenic germs be discovered. It is true that all the specimens yielded cocci, but in no case could any coli or coli-like bacilli be found. (Supplement to the 30th Report of L. G. B.)

It is also well known that many physicians occasionally order condensed milk for infants with satisfactory results as a temporary

measure, and certainly with no suspicion that there is any risk of inducing epidemic diarrhoea. Furthermore, an examination of the particular local milk supplies of the Croydon cases did not suggest that any particular dairies had any special incidence, or that distant dairies were more often marked out. Again, Chesterfield with a diarrhoeal rate of 54·4 per 1,000 during the years 1896 to 1899, derived 98 per cent. of its whole milk supply from cow-sheds situated within the borough or within a radius of five miles.

Therefore though hand-feeding is a very common antecedent of fatal diarrhoea, there is undoubted evidence that breast-fed children do sometimes suffer; there is further an absence of clinical evidence that *any large percentage* of diarrhoea deaths is due to commercial milk being infective when it reaches the homes. The commonest causes of diarrhoea should therefore be sought in the home or its immediate neighbourhood, though the preponderance of deaths among hand-fed infants shows that infection usually enters the body with the food.

No doubt dirty feeding-bottles may occasionally be infected from dirty milk and subsequently infect future meals, but if this were a common cause of fatal diarrhoea then these fatalities would probably be as common in the country as in the town, seeing that milk, as at present obtained, is probably even more dirty in the country than it is in the town.

It is hardly necessary to point out that there is no difficulty in explaining the infection of breast-fed babies. A few may be directly infected from previous cases, as in the exceedingly interesting series reported by Dr Bruce Low (supplement to Dr Ballard's report, *loc. cit.*), though this is probably uncommon[1]. More frequently infection could readily be derived from "matter in the wrong place" for which the human infant has such an astonishing predilection.

Seasonal incidence and meteorological relations.

It will be seen from Table III. that the well-known preference of summer diarrhoea for the third quarter of the year was marked both in each year and in the decennial average. This characteristic was especially marked in the years of exceptional prevalence.

The exact relations of diarrhoeal deaths in 1901 and 1902 are set forth in Charts I. and II. Unfortunately 4 ft. earth tempera-

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[1] These cases of communicable diarrhoea are probably *sui generis*—only one of Dr Low's series occurred in the summer quarter.

tures are not available before 1901. According to Ballard "the rise of diarrhoeal mortality does not commence until the mean temperature recorded by the 4 ft. earth thermometer has attained somewhere about 56° F., no matter what may have been the temperature previously attained by the atmosphere or recorded by the foot earth thermometer. The maximum diarrhoeal mortality of the year is usually observed in the week in which the temperature recorded by the 4 ft. earth thermometer attains its mean weekly maximum." Charts I. and II. show marked exceptions to this rule. In 1901 the 4 ft. thermometer reached 56° F. in the 27th week and attained its maximum in the 30th week. Yet diarrhoea deaths did not occur till the 30th week, and the maximum number of deaths was not registered till the 35th week. This is probably accounted for by the rainfall.

In the 27th week there were two days with good showers, amounting to ·5 and ·3 inches of rain; and the tendency for the diarrhoea deaths to increase in the 30th week was obviously checked by the five days in that week on which rain fell, while the four days on which rain fell in the 35th week had a similar effect.

In 1902 Ballard's rules were also departed from, though the relations to rainy days are fairly apparent.

A comparison between 1894 and 1902 is particularly instructive. In the third quarter of 1894 there were only 15 diarrhoeal deaths as compared with 56 in 1902.

Now the mean temperature of the air was very similar, though the diarrhoea mortality was very different. Mr Baldwin Latham has further been good enough to furnish me with figures for the mean monthly temperature of the soil at 2½ ft. and 5 ft. In July and August 1894 both these temperatures were higher than in 1902, though in September 1902 there was about 1° difference the other way. In 1894 however there were 56 days in the 3rd quarter on which rain fell as compared with only 42 days in 1902. The total rainfall in the 3rd quarter of 1894 amounted to 8·22 inches, while only 5·50 inches fell in the 3rd quarter of 1902. Neither the temperature of the air nor the temperature of the soil exhibited anything like this variation. These observations are in favour of Newsholme's contention (*Public Health*, Vol. XII. p. 163) that rainfall is probably a more important factor than temperature. They also suggest that the relation between diarrhoea and earth temperature is really quite indirect and simply means that the 4 foot thermometer expresses sustained heat modified by the cooling effect of showers. In other words, that the 4 ft.

YEAR 1901 CHART I

Shewing Deaths from
Diarrhoea & Enteritis

(Shaded Portion shew Enteritis)

Temperature above which
Diarrhoea is usually prevalent

Mean Temperature of Air

Mean Temperature
of the Ground at 4 feet.

Shewing Days on which Rain fell
(Days on which more than ½ inch fell
are shewn in Black)

YEAR 1902   CHART II

First Quarter	Second Quarter	Third Quarter	Fourth Quarter
Week Ending	Week Ending	Week Ending	Week Ending

Number of Deaths

Showing Deaths from
Diarrhoea & Enteritis
(Shaded Portion shows Enteritis)

Temperature above which
Diarrhoea is usually prevalent.

Mean Temperature of Air.

Mean Temperature
of the Ground at 4 feet

Temperature

Showing Days on which Rain fell.
(Days on which more than ·10 inch
fell are shewn in Black)

Days
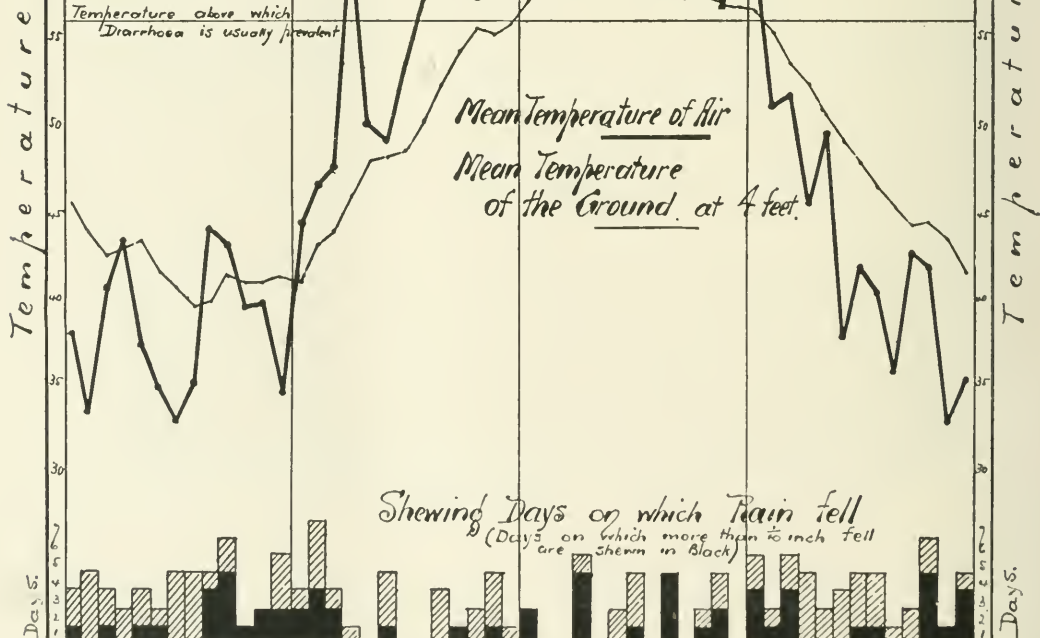
temperature compounds the existing influence of heat with the restraining effect of showers.

Whether this be so or not it is important to remember that in spite of loose talk about chills and the influence of climate *per se*, neither temperature nor rainfall can be to any appreciable extent the direct cause of fatal diarrhoea, seeing that they are only operative under urban conditions. For instance in the year 1899, though diarrhoea and enteritis were extremely prevalent in Croydon and accounted for 47·8 deaths per 1,000 births, there were only 9·3 deaths per 1,000 births in the neighbouring rural district of Godstone.

The returns for the Croydon Rural District are also of interest, and have been kindly obtained for me by the Medical Officer of Health, Dr C. M. Fegen. The Croydon Rural District is not only "suburban" in situation but in the literal meaning of that word. While much of the district is really rural, some of the townships and especially Mitcham are from a sanitary point of view urban in character. At any rate Mitcham has most of the disadvantages that are inherent to urban life. Now for the 10 years 1893 to 1902 the infantile diarrhoeal mortality in Mitcham was as much as 40·2 per 1,000 births, while the remaining portion of the Croydon Rural District had a rate of only 14·4 per 1,000 births.

Social status as a factor in Causation of Diarrhoea.

It is notorious that the incidence of fatal diarrhoea is greatest among the infants of the urban poor, though not by any means confined to the residuum.

Of the 974 deaths which occurred in Croydon during the decennium 1893–1902, 955 admitted of classification according to social status. Of these

409 occurred in infants of labourers;
532 occurred in infants of skilled artizans, clerks and tradesmen's assistants;
14 occurred in infants of the servant-keeping classes.

Why this should be so has not received an entirely adequate explanation.

Employment of mothers is no doubt a factor in some districts, as has been demonstrated in the case of Staffordshire by Dr Reid. But neither in Chesterfield nor in Croydon was this an important

factor. In Croydon for instance only 17 per cent. of the mothers of infants dying from diarrhoeal diseases were employed from home.

Others suggest that the high diarrhoeal mortality of towns is mainly an expression of lack of care and solicitude on the part of the parents and guardians of infants. No doubt this is to some extent true, as is shown by the high diarrhoeal mortality of illegitimate children. The idle, vicious and intemperate do gravitate to the towns, but diarrhoeal mortality is not limited to this class. Indeed if we neglect the residuum there seems not the least reason to suppose that rural mothers have a monopoly of either maternal affection, energy, thrift or cleanliness. We must therefore admit that the bulk of the difference between urban and rural diarrhoeal mortality is due to the fact that urban conditions render the care of infants, and hand-feeding in particular, more hazardous operations than they would be in rural districts.

There is no absolutely satisfactory way of demonstrating by statistics the share taken by social status in infantile mortality. Probably the best plan would be to draw up life tables for the infants of the "classes" and "masses" in some large town. At present however the necessary materials are wanting. In default the method suggested by Miss Collett, viz. comparison of the number of domestic servants per hundred families, is of value. This may be supplemented by comparison of the proportion of inhabitants occupying tenements of less than five rooms. These figures were therefore specially prepared by the Registrar-General at the recent census for certain wards and sub-districts in the borough.

The diarrhoeal rates for the decenniums are based on the following number of births in the various divisions.

West Ward	11,343
South Norwood Ward	4,714
South Ward	3,996
Central Ward	3,799
Thornton Heath Division	2,994
East Ward	2,832
Upper Norwood Division	1,252

TABLE V.

District	Diarrhoeal deaths per 1000 births 1893—1902	Domestic servants per 100 families	Percentage occupying less than 5 rooms	Percentage * overcrowded
Upper Norwood	15·1	95·8	20·3	1·73
South Ward	23·0	46·0	19·1	2·47
Thornton Heath	28·3	14·1	24·6	3·43
Central Ward	28·9	36·7	20·9	2·43
West ,,	32·6	21·7	23·4	4·52
East ,,	37·4	49·2	13·6	0·98
South Norwood Ward	37·7	30·5	11·6	1·17
Borough	31·1	34·3	19·3	2·74

* *i.e.* percentage living more than two to a room.

With regard to overcrowding of persons per room, it will be seen that the above table does not suggest any association of this condition with diarrhoeal deaths. Indeed, as far as can be gathered, diarrhoea is less often associated with overcrowding than are other infantile deaths, as was shown by inquiries at Croydon, where in the case of diarrhoeal deaths only 4 % of the tenements were overcrowded, as compared with 10 % in the case of respiratory and other diseases.

It is evident from Table V. that the variations in the diarrhoeal rates of the different parts of Croydon depend on other factors than the social status of the inhabitants, otherwise it would be impossible to explain why the East Ward with 49·2 servants per hundred families and only 13·6 per cent. of small tenement population should occupy such an inferior position to the Thornton Heath subdivision with only 14·1 servants per hundred families, and 24·6 per cent. of the population living in small tenements. Nor will social status explain why the South Ward comes off so much better than the East Ward, which as regards both percentage of servants and proportion of small tenement population has the advantage.

Can these anomalies be explained by considering the geological and physiographical difference of the various wards?

The geology of Croydon. The following brief description has been verified by Mr W. Whitaker, F.R.S., to whom I am indebted for much information and assistance on this and on many other occasions.

The subsoil in the north of the borough is London Clay, while the Upper Chalk comes to the surface in the south, the Clay and Chalk being separated by a strip of Lower London Tertiaries, comprised of beds of clay, sand, and pebbles. Both the London Clay and Chalk are in parts overlaid by irregularly disposed beds of gravel.

Taking the wards and subdivisions individually : *Upper Norwood subdivision* is practically all London Clay with some small superimposed beds of gravel—*Thornton Heath subdivision* is London Clay with some insignificant exceptions at the southern border including a small uprise of underlying sand near the railway—*South Norwood* is also London Clay—*East Ward* is varied, comprising London Clay, Blackheath Beds, Woolwich and Reading Beds, Thanet Sand and Chalk, with some drift gravel near the railway. Physically it varies considerably, clay, gravel, pebbles, sand, and chalk all being present—*The Central Ward* is almost entirely drift gravel overlying Tertiary Beds and Chalk—*The West Ward* also is mainly gravel, but with London Clay in its more northern and less built on portions, with an outcrop of Thanet Sand and Chalk at the southern end—*The South Ward* is largely on the Chalk, but drift gravel is found in the bottom of the valley on either side of the Brighton Road, where the smaller streets are situated, and Thanet Sand in a few streets near Duppas Hill.

Now the generally received opinion seems to be based on Ballard's dicta that solid rock is the least favourable to diarrhoea mortality, and that sandy soil is the most favourable : that clay on the whole is unfavourable, while gravel is favourable or unfavourable according as it approximates to sand or rock. It must be confessed that differences of subsoil do not afford any adequate explanation of the anomalies in the distribution of diarrhoeal mortality which have been already indicated. For instance Thornton Heath has the same subsoil as South Norwood, but a lower diarrhoeal rate in spite of adverse social conditions. The subsoil conditions in the poorer streets are in favour of the East Ward rather than the South Ward, though the diarrhoeal mortality is far lighter in the latter.

Physiography.

The Physiography of the various wards differs considerably :—

Upper Norwood subdivision is an elevated ridge of London Clay varying from 376 to 220 feet above O.D.; the smaller houses are situated in streets with very good slopes so that rainfall has its maximum effect in cleansing—*Thornton Heath subdivision* varies in elevation from 236 to 160 feet above O.D. The streets run for the most part down hill so that showers tend to cleanse the roads—*South Norwood Ward* varies from 200 to 140 feet above O.D.; but the part where diarrhoeal mortality is most marked has no great amount of fall, and what there is is discounted by the fact that the streets are more

often at right angles to the slope of the hill, and therefore do not benefit as much as they should from the scouring effects of heavy rain. The level of the subsoil water is very high and often at the surface in wet seasons. Many of the streets are not channelled—*The East Ward* exhibits physical as well as geological variations, but the poorer parts of the ward are nearly all on the flat with subsoil water near the surface—*The West and Central Wards* are flat, with little variation in altitude—*The South Ward* varies considerably in altitude. The poorer streets have for the most part a fair fall in the direction of their length.

It is here I believe that the essential local differences are to be found. Given equal social conditions and similar milk supply, the diarrhoeal mortality will, under equal meteorological conditions, vary with the surface conditions. If the surface contains an undue amount of injurious organic matter diarrhoea mortality will be high. If the surface has been well cleansed by artificial means, or scoured by rainfall, diarrhoea mortality will be low. On this hypothesis we can largely explain the local incidence of fatal diarrhoea in Croydon.

The mortality in the Upper Norwood subdivision is extremely low, not only on account of social conditions but because the poorer streets are to a large extent self-cleansing. This is also aided to some extent by the fact that many of the smaller streets are not thoroughfares and therefore do not suffer from disturbances by extraneous traffic.

Thornton Heath subdivision occupies a comparatively favourable position for a like reason—*South Norwood* and the low-lying portions of the *East Ward* come off badly because the roads in the particular parts inculpated are flat, not self-cleansing, and in some cases not properly channelled—*The South Ward* is better off than the East Ward for precisely opposite reasons. The poorer streets in the South Ward lie high and have good falls. Many of them also are not thoroughfares. In the East Ward on the other hand the poorer streets are almost dead level with the subsoil water very near the surface.

Do these physical advantages apply to the surface generally,
or only to the roads?

This is an important practical point which does not appear to admit of an answer at present. *A priori* it seems difficult to see how the physical advantages of rainfall acting on slopes could be extended to back gardens and unpaved yards, which are often enclosed by walls.

It is very possible that road sanitation is after all the main essential in limiting the toll from diarrhoea in urban districts.

*Other facts in favour of the importance of a dirty soil as a
predominant factor in the causation of diarrhoea.*

The figures for Croydon Rural District have already been quoted, and those for Mitcham confirm the probable importance of streets and courts which are not self-cleansing. From Dr Fegen's investigations it would appear that infantile diarrhoea deaths in Mitcham are confined to two localities, one on clay subsoil and the other on gravel. While differing from one another in subsoil, however, both agree in having a poor class population on a surface which is flat and not self-cleansing or readily cleansed.

Similarly, while Medical Officer of Health for Chesterfield, I was struck with the amazing rise in the diarrhoeal mortality during the years 1897, 1898, and 1899.

Chesterfield, it must be remarked, is almost entirely a privy midden town and therefore has all the disadvantages incidental to such a system. How very real these are and what an appalling amount of soil pollution results therefrom will hardly be credited by those who have no personal experience of the system. In the process of emptying middens night soil had always to be thrown on to the adjoining surface of either yard or back street. In many cases the excrement had then to be wheeled through an entry into the front street where the contractor would often deposit it in a heap while waiting for the collecting cart. Furthermore there was always more or less spilling of the refuse from wheelbarrows and carts owing to the work being done in the dark.

Now during the three years 1894 to 1896 there were only 51 deaths from diarrhoea in the summer quarter in infants under one year of age as compared with 136 such deaths in 1897 to 1899. No doubt much of this increased mortality was due to the climatic conditions being more favourable to diarrhoea in the latter period—for instance the summers were certainly drier—but one cannot but also associate the increase with the fact that in 1897 the local Sanitary Authority endeavoured to empty the middens more frequently and there were therefore many more opportunities for polluting the surface of the roads and yards. In 1894 to 1896, 4957 middens were emptied during June to September while 7,831 were emptied during the summer months of 1897 to 1899. Now the scavenging was at that time done by contractors, with anything but satisfactory results, and it is very suggestive that the rise in diarrhoeal mortality was most marked in that part of the town

where the work was done with least care and where the flatness of the surface allowed of little self-cleansing. The following are the figures:

	1894—6	1897—9	Increase
North	19	43	126 per cent.
South	20	35	75 ,,
West	12	58	383 ,,

It would be unwise to lay too much stress on these figures, but they certainly suggest that the only cure for the evils incidental to privy middens is wholesale conversion to the water-carriage system. More frequent cleansing may simply aggravate the evil by multiplying opportunities for surface pollution. This conclusion is supported by comparing the diarrhoeal rates for Chesterfield and Ilkeston, two neighbouring towns which are both situated on the coal measures and are very similar in size, industries and social status. From Dr Barwise's valuable report to the Derbyshire County Council for 1900 it would appear that the diarrhoeal rate per 1000 births for the ten years 1891—1900 was 37·7 in Chesterfield as compared with 17·8 in Ilkeston. Now refuse removal is done at the public expense in Chesterfield but at the expense of the owners in Ilkeston. This means that privy middens would be cleansed much less frequently in Ilkeston than in Chesterfield and that there is therefore less soil pollution of the grosser kind in the former borough.

There is also some reason to believe that diarrhoea is less common in streets and courts where there is no through traffic though this is difficult to prove in default of more exact information as to the distribution of births. At any rate it seems not unlikely, as Dr Waldo suggested, that horse manure may be an important source of soil pollution under favourable conditions.

How does polluted soil affect infants?

If it be agreed that diarrhoeal mortality has polluted soil for a frequent antecedent, it is still necessary to inquire by what process the human infant is injuriously affected by such a condition. Doubtless organisms capable of infecting infants are present in polluted soil, and these enter the infant's body by the mouth, usually along with the food. How these organisms are carried must still be an open question. Dust and the common house-fly would both be intelligible methods of conveyance. The frequently rapid effect of showers of rain rather

suggests the dust theory, as flies would rarely be killed by a few showers. The dust theory would also explain the incidence on the smaller streets where the houses are without front gardens and therefore more closely approximated to one of the sources of pollution. Fortunately the point is not of great practical importance, as in either case the removal of organic filth from the neighbourhood of houses is the first essential. When the filth is removed both soil pollution and flies will rapidly diminish. Personally however it is to the good offices of the road surveyor that I look with most interest and hopefulness. When we have roads, paths and passages rendered impermeable and properly swilled with water during the summer months we shall look forward with confidence to a diminution in summer diarrhoea and to increased health in other directions.

Lastly, it is necessary to explain why if diarrhoea is due to polluted roads and yards, it should be almost limited to the infants of the less affluent. It has already been pointed out that diarrhoea is largely a food infection and almost exclusively an urban phenomenon. It is common in the families of the less affluent for the simple reason that in towns scrupulous cleanliness of the infant's person, nursery, feeding-bottles and food is infinitely more difficult than under rural conditions. Scrupulous cleanliness means an expenditure in time and money that the hard-working wife of an artizan can hardly afford, and it is chiefly for this reason that municipal milk depôts appear to meet a want that a simple pure milk supply would not satisfy.

Conclusions.

(1) That fatal infantile diarrhoea is usually a form of food poisoning.

(2) That infection usually takes place at the home.

(3) That urban conditions are chiefly hazardous from the amount of polluted soil found in the roads and yards of urban districts.

(4) That infinite care is needed if babies are to be hand-fed in towns.

(5) That practical preventive measures should include:

(*a*) Impermeable roads with efficient channelling.

(*b*) Copious swilling of roads.

(c)   Education of mothers as to the necessity of scrupulous cleanliness.

(d)   The co-operative or municipal provision of specially prepared modified milk which should be sterilized during the diarrhoea season.

(e)   A more efficient control of the milk trade with special reference to the provision of cooled, approximately sterile milk from healthy cows.

(f)   The provision of houses which shall be sufficiently convenient to allow of their being cleansed with the least possible expenditure of energy.

A NEW LIFE-TABLE FOR ENGLAND
AND WALES.

(Constructed by an extended method.)

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Data.

When the publication of the series of Reports for each separate County, dealing with the facts ascertained at the Census of 1901, was completed last March, it was possible for anyone, who would take the trouble, to arrive at the facts relating to the whole of England and Wales, that is, to ascertain not only the finally corrected total population number, but the numbers classified into age and sex groups.

From the separate Annual Reports of the Registrar-General for the years 1891–1900, and for some preceding years, it was equally possible to compile the numbers of Deaths and of Births requisite for the construction of a new Life-Table.

While therefore it must be understood that the succeeding Tables have no sort of official sanction or guarantee, but are given on the sole responsibility of a private individual, it must also be understood that they are based on officially published figures.

Methods.

The mean population numbers, or "years of life," were calculated from the data of the Censuses of 1891 and 1901 by the method described by Mr A. C. Waters in the *Journal of the Royal Statistical Society*, Vol. LXIV., Part II., June 1901, p. 203.

In order to save space the actual numbers of "years of life" and of deaths in the respective age-groups are not given.

23—2

If reference be made to a series of papers contributed by the writer to the *Journal of Hygiene*, Vol. II., Nos. 1, 2 and 3, but little additional description will be required of the methods employed in the construction of the following Tables.

(1)　The $\log p_x$ values for ages 5 to 64 inclusive were obtained by the "graphic" process described in this *Journal* in Nos. 2 and 3 of Vol. II., the curve used being that described as "Section 1" in No. 3, page 206.

(2)　Although it has been shown that the same process might be employed for obtaining the succeeding $\log p_x$ values from age 65 onwards by drawing the curve in different sections and on different scales, the writer has found that it is easier and more expeditious, as well as more accurate, to obtain the $\log p_x$ values from age 65 onwards by a simple process of analytical interpolation. Space does not permit of going into full detail or giving actual illustrative calculations, but at the references already given it will be found how to calculate readily the values of $\log p'_x$ for the following values of x; $77\frac{1}{2}$, $82\frac{1}{2}$, $87\frac{1}{2}$, $92\frac{1}{2}$, and $97\frac{1}{2}$.

On setting down this series of values in a column and differencing them it is easy to obtain from the line of 5-yearly differences opposite to $u_{77\frac{1}{2}}$, (as u_0), the differences corresponding to intervals of one year, by the formulæ :

$$\delta^4 u_0 = \cdot0016\Delta^4 u_0$$

$$\delta^3 u_0 = \quad \cdot008\Delta^3 u_0 - 6\delta^4 u_0$$

$$\delta^2 u_0 = \quad\quad \cdot04\Delta^2 u_0 - 4\delta^3 u_0 - 8\delta^4 u_0$$

$$\delta u_0 = \quad\quad\quad \cdot2\Delta u_0 - 2\delta^2 u_0 - 2\delta^3 u_0 - \delta^4 u_0.$$

The series can then be carried upwards as far as u_{65} and downwards as far as may be required.

The values thus obtained are $\log p_x$ values, that is, they represent the chance of living from age x to age $x+1$, and they are ready to be used for the calculation of the l_x column of the Life-Table without any further alteration.

It has been found in each case that the two curves have joined each other fairly accurately at ages 64, 65.

In comparing different methods of constructing "extended" Life-Tables, attention must be concentrated upon the fact that the differences only apply to the calculation of the $\log p_x$ values.

Whatever method may be chosen, the labour of compiling the data and of calculating the l_x, P_x, Q_x, and E_x columns remains the same.

While admitting to the fullest extent the merits of the graphic method of distributing population and death numbers which is so ably advocated and illustrated by his friend Dr A. Newsholme, the writer is of opinion that from the points of view of economy of time and labour, as well as of accuracy, the *combined* method, herein briefly alluded to, is to be preferred.

An abstract of a Life-Table, constructed by an extended method, for ENGLAND
AND WALES, *based on the census enumerations of Population in* 1891 *and*
1901, *and on the recorded Deaths in the ten years* 1891—1900, *shown
in comparison with a Life-Table for the preceding decennium* 1881—90,
which has been recalculated by the same method.

[Columns (*a*) give the figures for 1891—1900, and columns (*b*) the differences of
these from the corresponding values for 1881—90.]

TABLE I.   *Males.*

Age x	Probability of surviving from age x to age $x+1$ p_x		Number of survivors at each age out of 100,000 born l_x		Mean expectation of life or after-lifetime at each exact age x E_x	
	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)
0	·82861	− ·01064	100,000		44·17*	+ 0·81
1	·94696	+ ·00415	82,861	− 1064	52·23	+ 1·63
2	·97922	+ ·00300	78,467	− 658	54·13	+ 1·49
3	·98685	+ ·00221	76,836	− 408	54·26	+ 1·35
4	·99033	+ ·00169	75,286	− 232	53·98	+ 1·25
5	·99298	+ ·00114	75,093	− 101	53·50	+ 1·17
10	·99770	+ ·00048	73,524	+ 253	49·60	+ 0·95
15	·99678	+ ·00043	72,631	+ 449	45·18	+ 0·84
20	·99541	+ ·00071	71,233	+ 586	41·01	+ 0·76
25	·99453	+ ·00084	69,502	+ 871	36·97	+ 0·62
30	·99312	+ ·00101	67,457	+ 1165	33·02	+ 0·48
35	·99083	+ ·00098	64,895	+ 1459	29·22	+ 0·33
40	·99835	+ ·00096	61,686	+ 1697	25·60	+ 0·19
45	·98503	+ ·00063	57,807	+ 1834	22·15	+ 0·10
50	·98044	+ ·00027	53,147	+ 1814	18·86	+ 0·05
55	·97391	+ ·00004	47,547	+ 1654	15·78	+ 0·05
60	·96465	+ ·00062	40,933	+ 1483	12·91	+ 0·03
65	·95008	+ ·00053	33,281	+ 1336	10·30	− 0·01
70	·92719	− ·00088	24,643	+ 992	8·02	− 0·02
75	·89368	− ·00164	15,778	+ 519	6·13	+ 0·04
80	·84748	+ ·00080	8,147	+ 223	4·64	+ 0·13
85	·78743	+ ·00957	3,103	+ 150	3·49	+ 0·21
90	·71356	+ ·02602	780	+ 112	2·64	+ 0·28
95	·62758	+ ·05095	113	+ 39	2·01	+ 0·29

* If the value of p_0 had been the same as it was for 1881—90 the value of E_0
would have been 44·73, or increased by 0·56.

TABLE II. *Females.*

Age x	Probability of surviving from age x to age $x+1$ p_x		Number of survivors at each age out of 100,000 born l_x		Mean expectation of life or after-lifetime at each exact age x E_x	
	(a)	(b)	(a)	(b)	(a)	(b)
0	·85963	− ·00945	100,000		47·82*	+ 1·04
1	·95061	+ ·00326	85,963	− 945	54·57	+ 1·80
2	·979J0	+ ·00286	81,717	− 615	56·38	+ 1·71
3	·98669	+ ·00180	80,074	− 368	56·52	+ 1·57
4	·99047	+ ·00147	79,008	− 218	56·28	+ 1·50
5	·99312	+ ·00122	78,255	− 99	55·82	+ 1·43
10	·99763	+ ·00050	76,613	+ 261	51·97	+ 1·21
15	·99683	+ ·00062	75,661	+ 438	47·59	+ 1·11
20	·99566	+ ·00099	74,268	+ 715	43·44	+ 0·96
25	·99511	+ ·00117	72,591	+1102	39·38	+ 0·75
30	·99383	+ ·00122	70,668	+1493	35·38	+ 0·54
35	·99197	+ ·00112	68,272	+1847	31·53	+ 0·36
40	·99044	+ ·00091	65,388	+2106	27·81	+ 0·21
45	·98833	+ ·00071	62,063	+2234	24·16	+ 0·12
50	·98474	+ ·00043	58,143	+2259	20·62	+ 0·06
55	·97938	+ ·00027	53,297	+2156	17·26	+ 0·03
60	·97123	+ ·00032	47,297	+1968	14·12	+ 0·02
65	·95814	+ ·00008	39,897	+1715	11·26	± 0·00
70	·93746	− ·00030	30,961	+1320	8·77	± 0·00
75	·90676	− ·00043	21,084	+ 855	6·70	+ 0·02
80	·86377	+ ·00041	11,810	+ 466	5·06	+ 0·06
85	·80688	+ ·00309	4,999	+ 235	3·79	+ 0·08
90	·73558	+ ·00820	1,436	+ 110	2·85	+ 0·11
95	·65098	+ ·01584	245	+ 36	2·15	+ 0·11

* If the value of p_0 had been the same as it was for 1881—90 the value of E_0 would have been 48·34, an excess of 0·52.

For the sake of experiment the p_x curve from age 5 to age 24 for England and Wales (males) for 1891–1900, has been calculated throughout by the writer, both by complete analytical interpolations in the series of log p'_x values, as already described in the *Journal of Hygiene,* and also by the method used for the new London Life-Table, and the results in each case almost exactly correspond with the series of values obtained by the graphic process which has been used for these Life-Tables.

Probably by a skilful use of Dr Newsholme's method results would be obtained almost the same.

Although the values of log p'_{10} and of log p'_{15} have been *calculated,* it has been found that there was no real need for this, as the values found by the empirical rules were practically identical.

If any reader should care to take the trouble to compare the newly calculated E_x values for England and Wales 1881–90 (which can be

arrived at from Tables I. and II. by means of the differences given) with the corresponding values given in the officially published Life-Table, it will be found that, proceeding upwards, they correspond with only differences of ± 0.01 until E_{35} is reached.

The differences found to exist from E_{30} to E_0 depend upon a different, and, as the writer believes, a more rational, system of interpolation having been employed for arriving at the values of p_5 to p_{34}.

Before these extended Life-Tables had been constructed the writer had already calculated the E_x and $E_{x \text{ to } x+n}$ values by the modified short method, and the results agree as closely as in the now considerable number of instances which have been previously tried, so closely, that but for the sake of obtaining accurate l_x values, the calculation of the extended Life-Tables might have been almost dispensed with.

Comments.

Space will only permit a brief allusion to the most important points.

(1) The mean rate of infant mortality for 1891–1900 having been considerably in excess of that for 1881–90, both as regards males and females, there is necessarily a considerable *inverse* difference between the respective p_0 values.

With this exception, and with the exception that for males the p_x values for ages 68 to 79 inclusive, and for females, those for ages 67 to 78 inclusive, which are *less*, all the other p_x values are *greater* than the corresponding ones for 1881–90.

(2) As regards the l_x columns it follows that as the p_0 values are lower, the l_1 values must also be less, and that the succeeding l_x values will also be less until the loss in the p_0 values is counterbalanced by the gain in the values of p_1......

The adverse balance is wiped off and transferred to the other side both for males and females, when l_7 is reached.

(3) As regards the E_x values, with the exception in the case of males that for ages 65 to 71 inclusive they fall fractionally below, and in the case of females that for ages 65 to 70 inclusive they coincide with the corresponding values of 1881–90, there is an increase all throughout, but the lowered values of p_0 have taken off both for males and females about half a year from the E_0 values.

(4) The comparatively small increase of average "Life-capital" shown in Table V. is due to altered proportional age-distribution of population.

TABLE III.

Showing how the total expectation of Life at Birth, (E_0), is on the average distributed over the main age-periods of life.

Age-periods	Males			Females		
	1881—90	1891—1900	Differences	1881—90	1891—1900	Differences
0—5	4·02	3·99	− 0·03	4·16	4·14	− 0·02
5—15	7·34	7·36	+ 0·02	7·65	7·67	+ 0·02
15—65	28·71	29·39	+ 0·68	30·67	31·52	+ 0·85
65 and upwards	3·29	3·43	+ 0·14	4·30	4·49	+ 0·19
Totals	43·36	44·17	+ 0·81	46·78	47·82	+ 1·04

TABLE IV.

Showing the average future lifetime or mean expectation of Life of all the individuals included within the age-groups indicated $(E_{x \text{ to } x+n})$.

Age-groups	Males			Females		
	1881—90	1891—1900	Differences	1881—90	1891—1900	Differences
0—5	51·36	52·74	+ 1·38	53·58	55·16	+ 1·58
5—10	50·63	51·69	+ 1·06	52·72	54·03	+ 1·31
10—15	46·47	47·39	+ 0·92	48·62	49·78	+ 1·16
15—25	40·32	41·06	+ 0·74	42·54	43·48	+ 0·94
25—35	32·62	33·09	+ 0·47	34·91	35·45	+ 0·54
35—45	25·50	25·70	+ 0·20	27·66	27·88	+ 0·22
45—55	18·94	19·00	+ 0·06	20·67	20·74	+ 0·07
55—65	13·09	13·12	+ 0·03	14·30	14·31	+ 0·01
65—75	8·35	8·34	− 0·01	9·08	9·08	± 0·00
75—85	4·93	5·03	+ 0·10	5·40	5·45	+ 0·05
85 and upwards	2·78	3·00	+ 0·22	3·12	3·21	+ 0·09

TABLE V.

Showing the average "Life-capital" of the census populations of England and Wales in 1891 and 1901, the figures having been obtained by applying the life-values of the preceding decennium, as given in Table IV., to the numbers enumerated in the respective age-groups at the following census.

	1891	1901	Differences
Males	36·09	36·15	+ 0·06
Females	37·44	37·61	+ 0·17
Parsons	36·78	36·90	+ 0·12

ADDENDA.

(1) The accuracy of the numbers of population compiled from the separate County Census Reports has been verified by their correspondence with the numbers given in the recently published *Summary Tables* of the census of 1901.

(2) If reference be made to the current number of *The Journal of the Royal Statistical Society*, a comparison may be made between the results given in the preceding Tables and those obtained by the "modified short method," and other Tables giving death-rates and mean age and sex distribution of population may be found.

(3) The writer desires to make acknowledgement of help received in the construction of the above Tables from Mr Maddison, Mining Surveyor of Haydock, in drawing and measuring the p_x curves, and from Mr Chas. Dickinson, Sanitary Inspector of Haydock, in making some of the routine calculations and in checking others.

THE BIOLOGICAL OR PRECIPITIN TEST FOR BLOOD, CONSIDERED MAINLY FROM ITS MEDICO-LEGAL ASPECT. II.

By G. S. GRAHAM-SMITH, M.A., D.P.H., M.B. (Camb.).

(*Continued from p. 291.*)

(*From the Pathological Laboratory of the University of Cambridge.*)

The Influence of Heat on (*a*) *Anti-sera, and* (*b*) *Normal Sera.*

(*a*) *Anti-sera.* The effects of heat on anti-sera have been investigated by many observers and all agree that a temperature of 60° C. is not sufficient to destroy their efficacy. Detailed observations as to the effects of temperature do not seem, however, to have yet been carried out. The following table summarises some of the observations on this subject:

	Destroyed at	Resisted		Observer
Haemato-sera	70° C.	65° C.	Weakened	Bordet (1899)
,,	—	60° C.	No effect	Rostoski (1902)
,,	—	60° C. for ½ hr.	Still effective	Obermayer and Pick (1902)
,,	65° C. for 24 hrs.	60° C. for 48 hrs.	Weakened	Linossier and Lemoine (1902)
,,	68° C. for 2 hrs.	52° C.	No effect	Michaëlis (1902)
Anti-egg serum		60° C. for 1 hr.	Scarcely affected	Uhlenhuth (1900)

In order to determine quantitatively the effects of heating on the precipitum-forming property, specimens of anti-sera were heated in small sealed capillary tubes attached to the side of a thermometer in a water-bath.

Specimens of anti-ox serum were heated for 5 minutes each, and of anti-sheep serum for 1·5 minutes, at the temperatures given in the following table. Subsequently ·1 c.c. of each sample was added to ·5 c.c. of a 1—21 dilution of its homologous blood, and the resulting precipitum measured quantitatively.

After the process of heating, no visible change was noticed in the anti-ox serum till a temperature of 65° C. was reached, when the fluid became slightly opalescent. At 70° C. this opalescence was very marked, and at 75° C. the serum became gray, opaque, and solid. In the case of

the anti-sheep serum slight opalescence was noticed at 66° C., which became more pronounced at 68° C. The following table shows that a marked reduction in the precipitum-forming power coincided with the visible change.

When the slightly opalescent anti-serum was added to a serum dilution a slight cloudiness appeared throughout the fluid. The more markedly opalescent serum differentiated itself as it settled to the bottom of the tube as a very definite cloud. After shaking the tube the fluid appeared cloudy throughout, but remained in this condition, no precipitum settling to the bottom.

The precipitum settled most quickly in the unheated specimens, and the rate of formation of precipitum decreased as the temperature, to which the anti-serum had been exposed, increased.

Up to 60° C. no change in the precipitum-forming power was found in either the anti-ox or anti-sheep sera, and both gave no trace of precipitum when heated beyond 67° C. Between 60° C. and 67° C. the quantity produced in each case was diminished. The figures given are the mean of two estimations in each case.

	Anti-ox (heated for 5 minutes)			Anti-sheep (heated for 1·5 minutes)		
Temp.	Precipitum	Percentage	Remarks	Precipitum	Percentage	Remarks
37° C.	·0234	100		·0075	100	
40	·0234	,,				
45	·0234	,,				
50	·0234	,,				
55	·0234	,,		·0075	100	
56				·0075	,,	
57				·0075	,,	
58				·0075	,,	
59				·0075	,,	
60	·0234	100		·0075	,,	
61				·0056	74	
62				·0056	,,	
63				·0065	83	
64				·0037	49	
65	·0187	79	Slight opalescence	trace	?	
66				,,	?	Slight opalescence
67	·0103	42	,, ,,	.	0	,, ,,
68				.	0	Marked ,,
69				.	0	,, ,,
70	.	0	Marked opalescence	.	0	,, ,,
75	.	0	Opaque, solid			

(b) *Normal Sera.* But few experiments seem yet to have been made on the effects of heat on normal sera in regard to their power of producing precipitum with their homologous anti-sera. Some of these experiments have been carried out on undiluted specimens, and others on

diluted, the results in the latter case not being strictly comparable with those in the former.

Serum	Destroyed at	Resisted		Observer
Eel	80° C.	58° C.	Less reaction	Tchistovitch (1899)
Fowl		70° C. for ½ hr.		Bordet (1899)
Diluted 1—100	100° C. 5 min.	55° C. ,, ,,	No effect	Nuttall (1901)
,, 1—10	65° C. 24 hrs.	60° C. for 4 days.	No effect	Linossier and Lemoine (1902)
Fowl's egg-albumin		56° C. for ½ hr.	No effect	Myers (1900)

The heating of specimens of undiluted ox serum (1 c.c. for 3 minutes), was carried out in the same manner as described for anti-sera. Subsequently 1—21 dilutions in salt solution were made, and tested with anti-ox serum.

No visible change in the serum was noticed till a temperature of 56° C. was reached, when the serum became slightly opalescent. This opalescence increased between 63—67° C., and was still further marked at 68° C. All these specimens gave slightly cloudy solutions. At 70° C. the serum became very opaque, and at 75° C. white and solid.

The quantity of precipitum formed remained constant up to 50° C., but from 55° C. to 62° C. a marked diminution was noticed. At 63° C. a further reduction occurred, and at higher temperatures the formation of precipitum ceased. All solutions gave a good foam-test.

The figures given below are the mean of two estimations in each case.

Normal undiluted ox-serum heated for 3 minutes.

Temp.	Precipitum	Percentage	Remarks	
Unheated	·0262	100		
40° C.	·0262	,,		
45	·0262	,,		
50	·0262	,,		
55	·0225	85		
56	·0225	,,	Slight opalescence	
57	·0215	,,	,,	,,
58	·0215	82	,,	,,
59	·0206	74	,,	,,
60	·0187	71	,,	,,
61	·0187	,,	,,	,,
62	·0187	,,	,,	,,
63	·0122	46	Increased opalescence	
64	·	0	,,	,,
65	·	0	,,	,,
66	·	0	,,	,,
67	·	0	,,	,,
68	·	0	Marked	,,
69	·	0	,,	,,
70	·	0	Opaque	
75	·	0	,, and solid	

These experiments, as far as they go, appear to indicate that an anti-serum can be exposed to a greater degree of heat than its corresponding serum without injury, and that the precipitum-producing property is completely destroyed in the latter at a lower temperature.

The effects of filtration of Normal Sera through "stone" filters.

It has been already shown (p. 279) that the substance of "stone" filters when allowed to act on serum exerts some influence on the serum exposed to it. In order to further test this point ox-serum was filtered through a new Berkefeld filter, and through a new clean Chamberland filter. After a certain quantity of serum had filtered through it was removed, and specimens from it diluted and tested. It was found that in the former case the precipitum-forming power was at first diminished, but returned to the normal after 110 c.c. had been filtered. No change was noticed during the passage of a further 300 c.c. through the filter.

In the latter case the precipitum-forming property diminished rapidly and fairly uniformly as the filter became choked.

Ox-serum.

Quantity filtered in c.c.	New Berkefeld filter		New clean Chamberland filter	
	Precipitum	Percentage	Precipitum	Percentage
Unfiltered	·0281	100	·0281	100
10	·0187	66		
20	·0210	74		
30	·0229	82	·0272	97
40	·0225	80		
50	·0229	82	·0272	97
60	·0229	82		
70	·0215	76		
80	·0225	80		
90	·0244	86		
100			·0245	87
110	·0225	80		·
125	·0281	100		
140	·0272	96		
150			·0158	56
165	·0281	100		
200	·0281	100	·0158	56
250	·0281	100	·0114	40
350			·0114	40
400	·0281	100		

The effects of the prolonged action of Antiseptics on Fluid Sera.

Unless fluid sera can be stored in a sterile condition it has generally been found desirable to add small quantities of antiseptics for the purpose of checking bacterial growth. In order to determine the effects of such antiseptics the following experiments have been carried out. Antiseptics in the proportions given below were added to fluid ox, and sheep sera, and allowed to act in sealed bulbs for 4 months. None completely checked bacterial growth. After this period dilutions of 1—21 in salt solution were made, and all were allowed to stand in open dishes for 2 hours in order that the volatile antiseptics should evaporate off. The results were compared with serum kept under the same conditions but without the addition of any antiseptic. The following table shows that in nearly all cases the precipitum-forming power was slightly reduced, but in a few completely destroyed.

The effects of the presence of these substances in fluids to be tested have been given previously (pp. 285—287).

		Ox serum		Sheep serum	
		Precipitum	Percentage	Precipitum	Percentage
Normal ox and sheep sera		·0356	100	·0140	100
Chloroform	1—1000	·0338	95	·0103	73
	1—500	·0328	89		
	1—100	·0187	55	·0093	65
Xylol	1—500	·0300	84		
	1—100	·0300	84	·0140	100
	1—25	·0281	79		
Benzol	1—1000	·0281	79		
	1—500	·0187	55		
	1—100	·0225	63	·0112	80
Toluol	1—100			·0140	100
Ether	1—500	·0347	97		
	1—100	·0244	68	·0084	60
Formalin	1—10,000	·0262	73		
	1—1000	·	0		
	1—500	·	0		
Alcohol	1—1000			·0075	53
	1—100			·0103	73
Lysol	1—500	·0187	55		
	1—100	·0169	47		
	1—25	·	0		
Lysoform	1—1000	·	0		
	1—100	·	0		
Chinosol	1—500	·0262	73	·0112	80
	1—200	·0262	73		

The effects of the prolonged action of Acids and Alkalis on Fluid Sera.

Ox, and sheep, sera with acids and alkalis added in the proportions given below were kept under conditions similar to those just mentioned. After dilution, the solutions were neutralized, and then tested quantitatively. Control antisera were also used in all cases, but gave no reactions.

The effects of the presence of unneutralized acids and alkalis have been given previously (pp. 281—285).

The following table shows that, except in very small quantities, the prolonged action of inorganic acids completely destroys the precipitatable substance, but that organic acids do not exert so deleterious an influence. Strong alkalis act in the same way as inorganic acids.

		Ox serum		Sheep serum	
		Precipitum	Percentage	Precipitum	Percentage
Normal ox and sheep sera		·0356	100	·0140	100
Hydrochloric acid	1—1000			·0093	65
	1—500	trace	?		
	1—100	·	0		
Nitric acid	1—1000			·0103	73
	1—500	·0046	13		
	1—100	·	0	·	0
Sulphuric acid	1—1000			·0112	80
	1—100			·	0
Acetic acid	1—1000			·0112	80
	1—500	·0244	68		
	1—100	·0300	84	·0112	80
	1—10	trace	?		
Oxalic acid	1—1000	·0225	63	·0140	100
	1—500	·0244	68		
	1—10			·0028	20
Carbolic acid	1—1000			·0131	93
	1—100			·0112	80
Citric acid	1—1000			·0131	93
	1—100			·	0
Caustic potash	1—1000			·0122	87
	1—500	·0150	42		
	1—100	·	0	·	0
Caustic soda	1—1000			·0122	87
	1—100			·	0
Sodium carbonate	1—1000			·0103	73
	1—500	·0244	68		
	1—100			·0103	73
	1—10	·0244	68		
Ammonia	1—1000			·0140	100
	1—500	·0309	84		
	1—100	·0206	57	·0131	93
	1—10	·0065	18		

In April 1901 Uhlenhuth (25. iv. 01) published the results of experiments on the reaction of human blood to this test under conditions

likely to be met with in medico-legal practice. Three months later he carried out investigations on materials obtained from the public prosecutors (25. VII. 01). It was not until after his experiments had been made, and his results transmitted to the authorities, that he was informed as to the identity of the blood-stains which he had examined. Lists of the articles examined, the methods employed, and the results arrived at are very fully given by him (25. IV. 01, 25. VII. 01, and VII. 02). In every instance his diagnosis as to the presence, or absence, of human blood was correct, and in many cases he even went further, and was able to correctly name the animal from which the blood was derived in cases in which the stain was not that of human blood.

The following case may be cited as an example of the efficacy of this test. The clothes of a man suspected of having committed a murder were sent to Uhlenhuth for examination. The accused was also suspected of having slaughtered some sheep in a field a fortnight before the murder. The tests revealed the following facts.

	Human blood	Sheep's blood
Coat	6 places	6 places
Trousers	7 ,,	3 ,,
Waistcoat	4 ,,	0 ,,
Shirt	1 ,,	0 ,,
Hat	4 ,,	0 ,,

The evidence in court subsequently made it absolutely certain that the accused had killed the sheep, and he was also convicted of murder and sentenced to death.

His procedure in making these investigations was as follows. Extracts of the stains were made in salt solution, and tested by chemical and spectroscopic tests in order to determine whether the stains were due to blood or other substances. About 4 c.c. of the extract, foaming readily on shaking, were placed in a test-tube and five drops of human anti-serum run in. A positive reaction to human anti-serum was shown by marked clouding within a few minutes. When this did not occur the test was concluded to be negative, and another anti-serum was added. This proceeding was repeated till a marked clouding appeared with one of the anti-sera. When no positive reaction was shown with any of his anti-sera he considered that the stain was due to the blood of some animal to which he possessed no anti-serum.

For example, in diagnosing a blood-stain on linen, given him by Prof. Beumer, he first added to the extract human anti-serum, then anti-sheep, and anti-horse, all with negative results. Finally he added

anti-pig serum, which gave a strongly positive reaction. The diagnosis of pig's-blood was confirmed by Prof. Beumer.

This method is open to the objection that a late clouding due to one of the anti-sera previously used may appear shortly after the addition of a subsequent anti-serum and give rise to a mistaken diagnosis. For this reason it would appear better to use smaller quantities in different tubes, and add a drop or two of an anti-serum to each tube.

Uhlenhuth always controlled his experiments by means of extracts of known dried bloods. In his papers he calls attention to the necessity in medico-legal investigations of proving the stains to be due to blood, and not albumen, or some other substance, by means of chemical and spectroscopic tests, and warns against the use of weak and opalescent anti-sera.

Ziemke (17. VIII. 01) made numerous experiments and demonstrated the possibility of differentiating human from other bloods, when dried on various materials. He frequently used soda solution (·1 %) as a solvent since he found that by this means he obtained a reaction when salt solution extracts gave none. The possibility of mistakes owing to the use of this solvent has already been mentioned (p. 283).

Whittier (18. I. 02) and Wood (24. IV. 02) have recorded instances in America in which the test has been made use of in legal cases. In both the presence of human blood was proved. The methods proposed by Nuttall were adopted. Austin (12. III. 03) in a paper on "the limitations of the Uhlenhuth test for the differentiation of human blood" showed that "other fluids of the human body, like effusions and exudates, were of little value" in the production of anti-sera. These facts, however, can have no bearing on the test in its medico-legal aspect, and but confirm the observations of others.

No attempt has been made to summarize the extensive literature on this subject, and these few citations are merely intended to emphasize the importance attached by foreign observers to this means of determining the origin of blood-stains in medico-legal investigations.

We must gratefully acknowledge the unfailing courtesy and kindness of Mr Henry, Chief of the Criminal Investigation Department of Scotland Yard, in placing at our disposal the unrivalled collection of medico-legal material in the Museum under his supervision.

Summary.

1. Powerful anti-sera may be produced by the intravenous injection of smaller quantities of serum than have hitherto generally been used (p. 260).

2. Nuttall's quantitative method affords a simple and fairly accurate means of determining the quantity of precipitum formed. By its means quantitative differences can be appreciated which are scarcely, or not at all, apparent in the tubes on inspection (p. 263).

3. Normal saline solution is the best diluent for normal sera, and 1—21 has been found to be a convenient dilution. Increase of salt has very little effect on the production of precipitum (p. 266).

4. The quantity of precipitum formed is not influenced by the temperature at which the experiment is conducted (*i.e.* between the temperature of the ice-chest and 37° C.) (p. 268).

5. In the case of dried bloods time *per se* does not destroy their capacity for reacting with their homologous anti-sera. Fluid sera appear to deteriorate slightly by keeping (pp. 269—274).

6. Putrefaction of the serum, or anti-serum, does not affect the production of a specific precipitum (p. 274).

7. Although the intimate mixture of lime and blood completely destroys the latter, the former is not present in sufficient quantity in ordinary earths to affect blood mixed with earth. The presence of small quantities of lime, however, gives rise to a clouding in solution, which can be got rid of by the passage of CO_2, and subsequent filtration (pp. 276—281).

8. The presence of even small quantities of acids, or alkalis, rapidly reduces the quantity of precipitum formed (pp. 281—284).

9. In diseased conditions a marked alteration may occur in the quantity of precipitum (pp. 265 and 285).

10. The volatile antiseptics produce little effect on sera, even after long contact, but formalin, corrosive sublimate, lysol, lysoform, the sulphates of copper and iron, and nitrate of silver, especially in strong solutions, exert a very deleterious action (pp. 287 and 358).

11. Blood dried on fabrics, and materials in common use (with the exception of certain leathers) may with adequate precautions be readily diagnosed (p. 290).

12. After an undiluted anti-serum has been raised to a temperature beyond 60° C. the capacity for producing precipitum is diminished, and

it is destroyed completely after exposure to 68° C. These effects seem to be produced at lower temperatures in normal undiluted sera (pp. 354–55).

13. The precipitum-producing power of normal sera is reduced by filtration through a Chamberland filter, but not by passage through a Berkefeld filter (as far as the experiment was conducted) (p. 357).

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ON THE CULTIVATION OF THE NITROSO-BACTERIUM.

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SINCE the time that Schloesing and Muntz[1] first discovered that ammonia could be converted into nitric acid through the agency of micro-organisms much attention has been paid to these bacteria. Many experiments were made by Warington[2] and P. and G. C. Frankland[3] to obtain pure cultures; but since they were only able to grow their micro-organisms in fluid media their results were not perfected. Winogradsky[4] has obtained more definite results by cultivating the micro-organisms both in fluid media and on plates. In growing the nitroso-bacteria Winogradsky employed a solution containing ammonium sulphate, potassium phosphate, and a basic carbonate, chiefly magnesium. Solutions made in this way were inoculated with soil, and when the ammonia showed evidence of oxidation sub-cultures were taken. He further isolated the bacterium from the sub-cultures by cultivation. As a result of his experiments he came to the conclusion that the nitroso-bacterium could not grow in the presence of organic matter, and that there are two distinct species of bacteria, morphologically similar, that can exist side by side in inorganic solutions. One of these, the nitroso-bacterium, can oxidise ammonia in an inorganic solution, but is unable to grow on agar, gelatine, or other ordinary medium, and dies in beef broth. The other species, although similar in form, does not oxidise ammonia, but can grow in inorganic solutions and thrives

[1] *Comptes Rendus*, vols. 84, 85, 86, and 89.

[2] *S. Chem. Soc.* Aug. 1888.

[3] *Proc. Roy. Soc. London*, vol. 47, S. 296, 1890.

[4] *Annales de l'Institut Pasteur*, nos. 4, 5, and 12, 1890; and *Archiv des Sciences Biologique*, St Petersburg, 1892.

on agar, gelatine, etc. In order therefore to isolate the nitroso-
bacterium he prepared plates from silica and certain inorganic salts
in solution, and by these means he was able to obtain good growth
of the bacterium in question in plates composed entirely of inorganic
constituents. In order to isolate the nitroso-bacterium by means
of organic media Winogradsky advised that particles of magnesia
taken from an oxidised ammonia tube be scattered on a gelatine
plate; any particles showing no growth would have a pure culture
of this species adhering to them.

In view of Winogradsky's work I commenced these investigations
in 1895. During the progress of my work there have been important
papers by Burri and Stutzer[1] criticising Winogradsky's results. In a
more recent paper Winogradsky, in conjunction with Omeliansky[2],
showed that the nitroso-bacterium is able to grow in the presence
of large amounts of organic matter.

The solution used by Winogradsky for growing the nitroso-bacterium
consists of water containing 1 per 1,000 ammonium sulphate, 1 per
1,000 potassium phosphate and 1 per 100 magnesium carbonate. The
carbonate solution is sterilised separately and added to the solution
of salts after sterilising to prevent chemical decomposition.

This solution I have continued to use all through the work, and
in this paper it will be referred to as the *ammonia solution*. It was
kept in test tubes, 10 c.c. being in each tube. It has always been
tested for the presence of oxides of nitrogen, and a control kept when
batches of the solution were inoculated.

The presence of nitrites is judged by a solution of diphenylamine
in sulphuric acid, Ilosvay's solution being used as a control when
necessary.

When I commenced the work I obtained cultures of the nitroso-
bacterium by inoculating ammonia solutions with small quantities,
0·2 g. or less, of various kinds of soil; rich garden soil, humus, sand,
etc. Tubes of the solution so inoculated were kept at room tem-
perature, and placed in a dark cupboard in order to avoid exposure
to light. The evidence of the growth of the nitroso-bacteria is found
in the conversion of the ammonia in the solution into nitrous acid, and
is the only one which I relied on in my work on these organisms,
although there is much evidence to show that this change does not
always take place even when good growth occurs. The conversion of

[1] *Centralblatt für Bakteriologie*, Abt. ii. Band 11, no. 4, 1896.
[2] *Centralblatt für Bakteriologie*, Abt. ii. Band 5, no. 12, 1899.

ammonia into nitrous acid is at first a slow process, and the chemical tests show that it does not as a rule commence for some 3 weeks, in tubes inoculated with soil; and having commenced, a week or two more are usually required before the whole of the contained ammonia is oxidised. Although this is a rule, many tubes have to be kept for months, and at times a year, before oxidation of the ammonia occurs.

I. *Original Cultures.*

I have inoculated ammonia solutions with various soils 43 times. Of these 43 solutions, 30, or 70%, oxidised, the majority within one month. One tube required four months before this occurred. The remaining 13 tubes did not oxidise during the time they were under observation, which extended to over a year in most instances. From tubes that showed oxidation of the contained ammonia, gelatine plates were poured; these showed moulds, yeasts, liquefying and non-liquefying bacilli; some species occurring on one culture and some on another. The only species constantly present on all the plates was an oval bacillus, morphologically like the nitroso-bacterium.

Dilutions from the original tubes.

In making sub-cultures I took 1/20 of a c.c. from the bottom of a culture tube. This had the two-fold advantage of excluding some extraneous organisms, and giving the smaller number of nitroso-bacteria a large amount of ammonia to oxidise. The comparatively few nitroso-bacteria present in a solution would result in some time elapsing before the ammonia was oxidised, and this length of time would tend to kill out most of the extraneous bacteria since there would be no organic matter present for them to feed on.

First Dilutions: 18 tubes were inoculated, and of these, 14 or 77% oxidised in 8 weeks. The remaining 4 tubes did not show any evidence of oxidation of the contained ammonia whilst under observation. Microscopic specimens made from the first dilution tubes that showed oxidation, showed chiefly oval bacilli corresponding in form to the nitroso-bacterium, rod-shaped bacilli being also present.

Second Dilutions: From the first dilution tubes seven sub-cultures were made. Six of these, or 85%, showed oxidation of the ammonia in 2 or 3 months. One tube failed to show oxidation whilst under observation. Microscopic specimens showed chiefly the oval bacteria as before, a few rod-shaped forms being also present.

Third Dilutions: Sub-cultures from the second dilutions were made in 10 instances. All of these oxidised the contained ammonia in 1 month. Microscopic specimens corresponded to those prepared from the second dilutions.

Fourth Dilutions: 14 sub-cultures were made from the third dilutions; of these 13, or 93%, showed oxidation. 11 oxidised the ammonia in 1 month : 2 oxidised

the ammonia in 3 months; 1 showed no evidence of oxidation. Microscopic specimens showed the oval bacteria in almost pure culture.

Fifth Dilutions: 9 sub-cultures were made from the fourth dilution tubes. Of these 7, or 77 %, showed oxidation. 6 oxidised the ammonia in 2 months. 1 oxidised the ammonia in some months; 2 showed no change. Microscopic specimens from these solutions showed the oval bacteria in almost pure culture.

Cultures of the Nitroso-Bacterium from Thames Water.

15 tubes which contained the ammonia solution were inoculated with small quantities of unfiltered Thames river water. Of these, 10 showed oxidation of the ammonia in from 1 to 2 months. The other 5 tubes exhibited no evidence ,of oxidation.

From the oxidised tubes 6 sub-cultures were made; but these gave no evidence of oxidation after being kept for periods of from 2 to 15 months.

No further experiments were made.

Ammonia solution, containing no carbonate, used as a medium.

In order to observe the effect of the absence of carbonate I prepared a solution containing 1 per 1,000 ammonium sulphate and 1 per 1,000 potassium phosphate but containing no carbonate. Four tubes of this solution were inoculated with strongly nitrifying cultures. These four tubes all showed oxidation of the contained ammonia in 10 weeks.

From these cultures sub-cultures in the same medium were made ; these showed no evidence of oxidation during the 5 months that they were under observation.

These experiments are interesting as showing that a certain amount of nitrification can occur without a basic carbonate being present, but this change cannot continue indefinitely. After a time the action ceases, and for continuous nitrification the carbonate is required.

A solution containing 1 in 10,000 ammonium sulphate and potassium phosphate used as a medium.

Experiments were made with an ammonia solution containing only 1 in 10,000 parts of ammonium sulphate and potassium phosphate, a fair quantity of magnesium carbonate being present. Several tubes of this solution were inoculated with strongly nitrifying cultures, but in no instance did oxidation occur during the several months in which the tubes were under observation.

Absence of nitrification was probably due to the small quantity of phosphates present.

II. *Liquid Media containing organic matter.*

I made a series of experiments with ammonia solutions containing various quantities of peptone beef broth, Witte's powdered peptone, and urea. Each of these substances was added to solutions containing the usual amount of ammonia, and potash phosphate and magnesium

carbonate, and the solutions were tested from time to time for the presence of nitrites.

Peptone Beef Broth.

The beef broth was added to the ammonia solutions in quantities varying from 1 in 11,000 to 10 per cent.; controls being kept in all cases.

1 *in* 11,000 *beef broth:* Six tubes were inoculated, one with earth, the other 5 with strongly nitrifying solutions. The tube inoculated with earth showed oxidation in 6 weeks. The 5 tubes inoculated with strongly nitrifying cultures oxidised in 2 months. Three sub-cultures all oxidised after some months. Control tubes showed no oxidation.

1 *in* 5,000 *beef broth:* Three tubes of this solution were inoculated. No. 1 with soil, No. 2 with a strongly nitrifying culture, No. 3 with a 1 in 11,000 beef broth tube that had oxidised. No. 1 oxidised in 12 months, No. 2 oxidised in 9 months, No. 3 oxidised in $2\frac{1}{4}$ months. The control tube showed no change.

1 *in* 2,000 *beef broth:* Two tubes were inoculated from the solutions oxidising in 1 in 5,000 beef broth. These both showed oxidation of the contained ammonia in 9 months. A control tube showed no oxidation.

1 *in* 1,000 *beef broth:* Two tubes of this solution were inoculated from 1 in 5,000 beef broth oxidising solution. These both showed oxidation of the contained ammonia in $2\frac{1}{2}$ months. Control tube showed no oxidation.

1 *in* 500 *beef broth:* Two tubes of this solution were inoculated from 1 in 1,000 beef broth oxidising solution. These showed oxidation of the contained ammonia in 6 weeks. Control tube showed no oxidation.

1 *in* 100 *beef broth:* Two tubes of this solution were inoculated from 1 in 1,000 beef broth oxidising solution. These showed oxidation of the contained ammonia in $5\frac{1}{2}$ months.

1 *in* 10 *beef broth:* Two tubes of this solution were inoculated from 1 in 1,000 beef broth oxidising solution. Directly after inoculation one of the tubes showed a very faint nitrite reaction, the other none. Both solutions showed good oxidation of the contained ammonia in $5\frac{1}{2}$ months. Control tubes showed no change.

Ammonia Solution containing Witte's peptone used as a medium.

1 *in* 11,000 *peptone:* Five tubes containing this solution were inoculated from strongly nitrifying cultures.

Results: 1 tube showed oxidation of contained ammonia in 1 month ; 1 tube showed oxidation of contained ammonia in 2 months ; 2 tubes showed oxidation of contained ammonia in 5 months ; 1 tube showed oxidation of contained ammonia in 12 months. 2 Sub-cultures obtained from robust nitrifying cultures oxidised in 1 month.

1 *in* 5,000 *peptone:* 9 tubes inoculated ; 5 with soil and 4 from 1 in 11,000 peptone solution that showed oxidation.

Results from soil: 2 tubes showed oxidation in $1\frac{1}{2}$ months ; 1 tube showed oxidation in 2 months ; 1 tube showed oxidation in 3 months ; 1 tube no change.

Results from 1 in 11,000 peptone: The 4 tubes inoculated showed oxidation in

2 months. Sub-culture showed oxidation of the ammonia in 9 months. Control tube showed no change.

Ammonia Solution containing Urea used as a medium.

1 *in* 11,000 *urea:* 7 tubes of this solution were inoculated, one with soil and 6 from strongly nitrifying solutions. The tube inoculated with soil showed oxidation in $1\frac{1}{2}$ months. The 6 tubes inoculated from the nitrifying solutions showed oxidation in periods varying from 1 month upward.

1 *in* 5,000 *urea:* 8 tubes inoculated. 4 with soil and 4 from the 1 in 11,000 urea solution that showed oxidation of the ammonia.

Results from soil: 3 tubes showed oxidation in 2 months; 1 tube showed oxidation some time later.

Results from 1 *in* 11,000 *urea solution:* 1 tube showed oxidation in $1\frac{1}{4}$ months; 2 tubes showed oxidation in 2 months; 1 tube showed oxidation some months later.

3 sub-cultures from tubes actively nitriting oxidised in 9 months. Control tube showed no oxidation.

1 *in* 2,000 *urea:* 3 tubes of this solution were inoculated with 1 in 5,000 urea solution showing oxidation. Of these three 1 showed oxidation in $2\frac{3}{4}$ months. The others showed no change in 10 months.

1 *in* 1,000 *urea:* 2 tubes containing this solution were inoculated with 1 in 5,000 urea solution that showed oxidation. No change was observed at the end of 10 months.

III.   *Experiments with Solid Media.*

Plate Cultures.

In carrying out the work of isolation of the nitroso-bacterium by plate culture I have worked with silica, gelatine, and agar plates.

Although for clearness of description I shall describe the silica, agar, and gelatine plates separately I was working with all three at one and the same time.

Silica Plates.

The following is the method adopted by Winogradsky in preparing silica plates:

Sodium silicate, or waterglass, is diluted with water until its specific gravity is 1·05. To 100 c.c. of this solution, 50 c.c. of hydrochloric acid specific gravity 1·1 are added, and this mixture is then poured into a dialyser. The dialyser containing the solution is kept in running tap-water for one day, it is then removed from the tap-water and placed in distilled water for two days, this distilled water being frequently renewed. The presence or absence of chlorides is noted by the reaction with silver nitrate solution. After the chlorides have almost entirely disappeared the solution is removed from the dialyser and concentrated by heat to about half its volume, and its setting power tested. This dialysed silica solution is then mixed

with a solution of various inorganic salts which is used as a culture medium. The coagulation of the silica in this solution forms the silica plate.

The solution of salts used consists of:

Ammonium Sulphate	...	0·4 gramme
Magnesium Sulphate	...	0·05 ,,
Potassium Phosphate	...	0·1 ,,
Calcium Chloride ...	...	a trace
Sodium Carbonate	...	0·6 to 0·9 gramme
Distilled Water ...	...	100 c.c.

A solution of the sulphates and chlorides, and another of the phosphates and carbonate are made and sterilised separately, and mixed when cool. The solutions are kept separate in order to avoid chemical change.

The solution of salts that is mixed with the silica solution serves two purposes. In the first place it aids in producing coagulation of the silica in solution. It also serves as a food for the nitroso-bacterium.

In endeavouring to prepare silica plates by this method many difficulties were encountered and it was months before I obtained plates that would grow the nitroso-bacterium. The chief difficulties that I met with were due to one or other of the following causes :

Using crude silicate of soda solution (commercial); preparing a pure silicate of soda in the laboratory ; leaky dialyser; using too strong solutions, either of silicate of soda or of hydrochloric acid ; mixing the acid and the silicate of soda solution in the wrong order ; exposing the dialysed silica solution in a large mouthed vessel to the atmosphere of the room.

The first of these difficulties can easily be obviated by using pure silicate of soda solution. The second I endeavoured to overcome by making pure sodium silicate in the laboratory. This I found to be a difficult and tedious process and liable to the error of introducing too much sodium carbonate in its manufacture. I therefore came to the conclusion that it was better and simpler to buy pure sodium silicate solution from a good firm, in spite of the drawback of having to wait a month or more for it (it cannot be obtained from English firms apparently). The difficulty with a leaky dialyser is usually avoided if great care is taken in testing the dialysing paper in the first place. Yet in spite of every care a leak will at times occur ; this is found out when testing the water for chlorides, silica giving a marked cloudiness in the presence of nitrate of silver, which clears up on addition of nitric acid. Using too strong a solution of either silicate of soda or hydro-chloric acid causes premature coagulation. If the solutions be very much too strong immediate coagulation occurs, if slightly too strong coagulation occurs in the dialyser. This can be avoided by using solutions of proper strength. If the silicate of soda solution be added to the acid, premature coagulation occurs; the acid must be added to the silicate of soda solution. If the dialysed solution be poured into a beaker and left, merely covered over for a few hours, before the salts have been added, it will coagulate spontaneously, through the CO_2 present in the air of the room acting on the large surface exposed, I believe. It is therefore necessary to keep the dialysed fluid in a narrow-necked vessel, and to use it as soon as possible.

In my earlier experiments I had to contend against each and all of these

difficulties, and the outcome of my experience was that I found it necessary to slightly alter the method advocated by Winogradsky.

The method eventually adopted was as follows : I took pure silicate of soda of a specific gravity somewhat less than that advised by Winogradsky ; its strength varied from 3·3 to 4%; to this I added an equal quantity of hydrochloric acid solution, specific gravity 1·1. It is, as before mentioned, necessary to add the acid to the silica, otherwise premature coagulation occurs. The silicate of soda solution and acid thus mixed were then dialysed. I dialysed the solution for 4 days in running tap-water in order to remove the chlorides, present as chloride of sodium, in the solution. It is not absolutely essential to remove all the sodium chloride, since this salt is not in any way prejudicial to the growth of the nitroso-bacterium if only present in small quantity, but I have found it preferable to remove it from the silica solution at this stage since any quantity above a trace would tend to produce spontaneous coagulation of the silica. After the greater portion of the chloride had been eliminated by dialysing in tap-water, the dialysis was continued with distilled water until all traces of chlorides had disappeared. After this process had been completed the dialysed fluid was poured into a narrow-necked flask and evaporated down to about half its bulk. When it had been concentrated to this extent it was ready to receive the salts, which were added to afford an inorganic means of sustenance to the micro-organisms. The solution of salts used for this purpose was the same as that advised by Winogradsky.

To prepare a silica plate the evaporated silica solution is pipetted off into a fairly deep Petri dish, and about half the quantity of the solution of salts added. The whole is then evaporated slowly over hot water until the silica coagulates and forms a clear whitish jelly-like mass with a smooth surface. The plate is then ready for inoculation. To inoculate such a plate the culture is first mixed with sterile water and then pipetted over the surface of the silica plate and any excess of the inoculating fluid poured off.

Growth of Organisms on Silica Plates.

The plates were inoculated with the following cultures :—

1. Ammonia solution which oxidised directly from soil (impure cultures).

2. Cultures containing few if any species of extraneous organisms, these having been excluded by dilutions and plate cultivation.

3. Cultures obtained from silica plate colonies (pure cultures).

Plate from impure culture.

From an impure culture one silica plate was inoculated. After eleven days colonies of various species were seen, these included two kinds of bacilli ; also yeasts and moulds. From this result I found at once that a medium containing only inorganic materials did not exclude or prevent the growth of all organisms except those which I sought, namely, the nitroso-bacterium ; and this fact at first created some doubts as to the possibility of my being able to obtain a pure culture of such

species by this means. Seeing that I must work from purer cultures I continued to inoculate silica plates from solutions in which most of the extraneous bacteria had died out.

Plates from 2nd Group of Cultures.

Several plates were inoculated. After 7 days at room temperature numerous tiny colonies were seen which looked like points and were almost invisible to the naked eye. Under the A. A. Zeiss these colonies were seen to be yellow or brown in colour, homogeneous in appearance, variable in size, with serrate or dentate margins. A "Klatsch," or contact specimen, showed that these colonies were made up of oval organisms.

All the plates inoculated showed great numbers of these micro-organisms. In half the plates they were in pure culture. In two a few bacilli were also seen surrounding the oval forms and forming with these latter a colony. A large oval organism was also noted in some one or two plates associated with the small oval bacillus. All the silica plates inoculated from the second group of cultures showed oxidation of the contained ammonia. In two instances this occurred in 12 days.

Plates from 3rd Group of Cultures.

Silica plates were inoculated with ammonia solutions that oxidised from colonies taken from earlier plates. After some days the colonies developed, and corresponded to those already described. A contact specimen showed a pure culture of oval bacilli corresponding to those found on the earlier plates.

From the silica plates that showed oxidation of the contained ammonia pieces containing colonies were on 9 occasions inoculated into ammonia solutions. Of these solutions 5 oxidised, one in 1 month, two in 2 months, one in $2\frac{1}{2}$ months, and one in 3 months. Several ammonia solutions were also inoculated with single colonies from silica plates which showed oxidation of the ammonia. One such ammonia solution showed oxidation. From this oxidised solution bouillon agar plates were poured and these plates showed good growth of colonies. On the original plate they were tiny, and under the A. A. Zeiss they were seen to be yellowish-brown in the centre with ground-glass-like margins. Their outline was regular. The dilution plates showed cream-coloured colonies varying in size up to 4 mm. in diameter. A microscopical specimen showed oval micro-organisms morphologically like the nitroso-bacterium.

Gelatine Plates.

The gelatine that was chiefly used was prepared from beef broth in the usual way. Besides this a gelatine medium was also prepared from extracts of various soils, these soil extracts being filtered and sterilised, and then gelatine to 10 % added. Neither peptone nor sodium chloride was added, and the reaction was not altered unless it was acid to litmus. At first gelatine plates were poured and allowed to set; then particles of magnesia were taken from ammonia solutions that had oxidised, and scattered on them, as advised by Winogradsky, in the hope that one or other of these particles would show no growth and so yield a pure culture of the nitroso-bacterium. In no case however did I meet with this result, invariably numerous colonies developed round the particles of magnesia. The colonies that developed round the particles were often in nearly pure culture, and I noticed that they were made up of oval organisms, morphologically resembling the nitroso-bacterium. Secondly, I poured several plates from oxidised ammonia solutions. Those inoculated from the ammonia solutions oxidising directly, those from earth usually liquefied. Those plates inoculated from subcultures from ammonia solutions developed very numerous colonies, which produced no liquefaction. These colonies corresponded to those that grew round the particles of magnesia. To find if these colonies had any relation to the organisms I was in search of, small pieces of gelatine on which colonies were observed, were cut out by means of a platinum needle and inoculated into test-tubes containing the ammonia solution. Out of 8 tubes so inoculated one showed signs of oxidation of the ammonia at the end of 3 months. From this oxidised ammonia solution plates were poured, and these exhibited the same species of organism as already noted.

To the naked eye the colonies presented at first a whitish and polished appearance. After keeping the plates some days these colonies became pale yellow, and this colour deepened later on. Under the A. A. Zeiss they were seen to be irregular in shape, with a brown centre and white margin.

Gelatines prepared from diverse soils gave the same result, namely, a pure culture of an oval organism morphologically similar to the nitroso-bacterium.

Agar Plates.

With this medium, as with gelatine, I commenced by using agar prepared with beef broth.

In continuing my researches, beef broth was replaced by sterile soil extracts, and finally agar was prepared with the solution of salts which I had always used as a cultivation medium, namely, a solution consisting of 1 % ammonium sulphate, 1 % potassium phosphate, and 1 % magnesium carbonate in distilled water.

Beef broth agar was inoculated with ammonia solutions that had oxidised directly from soil. These plates showed moulds, yeasts, and various bacilli, numerous oval forms being present with others.

A large number of agar plates were then poured from subcultures of the oxidised ammonia solutions. These plates showed in all cases a large predominance of an oval micro-organism, and in many instances a pure culture of this species. It corresponded to that already seen on the gelatine plates. After 6 days at room temperature the colonies appeared to the naked eye as white, iridescent growths varying in size. Some days later they became lemon coloured, and later yellow. Under the A. A. Zeiss the colonies were seen to have a brown centre, the colour fading at the margin.

Beef broth agar plates were also poured from the solution that had oxidised from the piece of gelatine plate containing colonies, already mentioned. These plates gave precisely the same result as above.

The further experiments made with agar were carried out in the hope of proving that this was either the nitroso-bacterium or a parallel organism which was morphologically similar but physiologically different. To this end beef broth agar plates which showed a pure culture of the oval organisms were taken, and pieces containing these colonies were inoculated into sterile ammonia solutions. 53 such tubes were inoculated and 20 showed oxidation of the ammonia, after two months as a rule.

Since these pieces of agar plates so inoculated might have produced oxidation from nitroso-bacteria which were present but not growing, control experiments were made by inoculating beef broth agar plates with solutions which would produce a pure culture of the colonies of the oval bacillus. These plates were then kept until the colonies had developed, and pieces of the agar removed where no colonies were seen after careful search with the microscope, and were inoculated into sterile ammonia tubes and kept for a period of from 10 weeks to 4 months. This experiment was made 19 times but on no occasion was any oxidation set up in the ammonia solutions inoculated. Hence we have :

Agar plate with colonies : Inoculated 53, Oxidised 20.
Agar plate without colonies : Inoculated 19, Oxidised 0.

As before mentioned, agars were also prepared from various soil extracts. Among these may be mentioned watery extracts from garden soil, watery extract from old manure heaps, watery extract from Thames mud ; and these together with any other variety of soil obtainable were used to prepare agars, which I hoped and trusted would form a suitable means of growing the nitroso-bacterium.

These agars yielded precisely similar results to those already mentioned.

Being unsuccessful in obtaining oxidation of the ammonia from a single colony taken from bouillon agar I took a single colony from

a silica plate and grew it on sloping bouillon agar. When I had obtained good growth I subcultured it into an ammonia solution that was in an artificial filter containing sterile soil.

This filter consisted of two glass cylinders each about 1 inch in diameter, plugged at one end with wool and at the other end with perforated india-rubber corks. Through the perforation in these corks glass tubes were fitted and connected by means of a piece of india-rubber tubing about 8 inches in length. The cylinders were thus connected in such a way that not only could fluid pass from one cylinder to the other, but either cylinder could be raised or lowered at pleasure. Into one of these cylinders, sterile soil to the depth of $1\frac{1}{2}$ inches was placed, and on to this soil the ammonia solution was poured in sufficient quantity to saturate the soil and also allow of the presence of about 50 c.c. of the fluid, which would either remain above the soil, or on raising this cylinder would flow through the soil into the other, empty cylinder. The whole filter was then sterilised. The cylinder containing soil and ammonia solution was then inoculated with the agar culture before mentioned. Every day the cylinder which contained the soil was either raised or lowered. By raising this cylinder the fluid would flow from the soil into the empty cylinder, leaving the soil in a condition that would allow of its aeration. On lowering the cylinder the fluid would flow back into the soil again. This was done daily and the soil thus alternately aerated and moistened without being exposed to contamination from extraneous organisms.

The ammonia in solution in this filter was oxidised in 10 weeks, a control filter showing no change. No other experiments were made after this manner on this occasion.

The plates which I have been most successful with in growing the nitroso-bacterium were prepared from ammonia agar. The medium consisting of ammonium sulphate, 1 g.; potassium phosphate, 1 g.; distilled water, 1 litre.

The salts were dissolved and agar added to $1\frac{1}{2}$ %, the whole boiled up and prepared as ordinary agar. After sterilising the agar some sterile carbonate of magnesia was added, the amount being roughly 1 % by weight, the exact amount being of no consequence.

As will be seen, this agar corresponded in composition to the ammonia solution used for the ordinary cultures, save for the presence of the $1\frac{1}{2}$ % agar. It has a slightly lower melting and coagulation point than bouillon agar.

Although this ammonia agar was the medium that I have been most successful with, yet it had certain disadvantages. The presence of the particles of carbonate of magnesia added to the difficulty in examining the plates, the colonies being closely associated with it and assuming a somewhat similar appearance under the A. A. Zeiss.

On this medium also the colonies of the nitroso-bacterium were very difficult to pick up; when a platinum needle was dipped into them they broke up, and neither specimen nor culture could be satisfactorily obtained. In order to be successful the colony had to be entirely removed by digging it out.

Having melted the agar and allowed it to cool to as low a temperature as was practicable, I inoculated 3 tubes in the usual way to obtain an original and two dilution plates. The culture that was inoculated was either a few drops of an oxidising ammonia solution or a piece of ammonia plate which contained colonies that had already undergone this process. On such plates the colonies developed in a few days at room temperature, and good oxidation of the ammonia could occur in as short a time as 3 weeks on the original plate. The dilution plates at times also showed nitrification a few weeks or months later, but in these plates the process was naturally much slower, when it did occur, on account of the fewer nitroso-bacteria present.

Inoculation of strongly Nitrifying Solutions.

This was done on 20 occasions. Of these plates so inoculated 16 original plates showed oxidation. The time varying from 3 weeks to 3 months.

The ammonia in the dilution plates oxidised less readily, and only 3 out of 40 poured showed this change.

Inoculation of small pieces of Ammonia Plates that showed oxidation.

This was repeated 35 times. Of the plates so inoculated 15 showed oxidation of the ammonia in from 1 to 2 months. From the oxidised ammonia agar plates pieces containing colonies were placed in ammonia solutions. After a lapse of time these solutions showed oxidation of the ammonia, and then from them ammonia agar plates were inoculated. This was done 6 times, dilution plates being also poured. All the original plates in these 6 experiments showed oxidation of the ammonia in from $1\frac{1}{4}$ to $4\frac{1}{4}$ months. None of the dilution plates showed any oxidation, although the same colonies could be observed on them.

Description of Colonies occurring on ammonia agar plates.

The plates were opened as a rule 1 month after inoculation, not sooner, to avoid the drying up of the medium.

In the plates that showed oxidation of the contained ammonia the colonies were numerous. They occurred in two forms, those which grew on the surface, and those in the depth. The colonies on the surface could not be seen by looking directly on to the plate, but if it were held almost on a level with the eyes they appeared as dull, colourless ground-glass-like growths 1 mm. or more in diameter. Under the A. A. Zeiss they could be seen by careful arrangement of light and focussing to be finely granular colonies with a dentate or almost moss-like margin. The colonies in the depth could only be seen as points to the naked eye. Under the A. A. Zeiss they exhibited the following characteristics, the centre had a flaky or lumpy appearance, and was of a pale reddish-brown colour; from this centre the colony

spread indefinitely and almost invisibly, its colour being lost, only a faint brown shade and a granular appearance was seen; this spreading margin extended in some instances to a neighbouring colony. At times the granular margin assumed a filamentous character, giving the colony a spider-like appearance. The very tiny colonies had the same flaky or lumpy appearance, but one could not see any spreading margin; their outline appeared to be more definite and regular.

Specimens made from all the above forms of colonies showed micro-organisms morphologically similar to the nitroso-bacterium.

All the ammonia agar plates that showed oxidation contained great numbers of them. The plates which showed no oxidation contained few or none, or else (as was seen on one or two occasions) they were very poorly developed all over the plate.

I also endeavoured to inoculate single colonies on sloping ammonia agar in tubes, but of several tubes so inoculated one only showed good oxidation of the ammonia; this occurring in 9 months. From the centre of this tube, which showed oxidation of the ammonia through its whole bulk, a tiny piece was taken and inoculated into beef broth agar, and plates poured. In 3 days at room temperature enormous numbers of colonies, some 3,000,000 or more, were seen under the A. A. Zeiss. They were sherry coloured, and had a sharp outline but no definite shape. The dilution plates showed greyish-white, polished, semi-translucent colonies which after some days developed to 1 mm. or more in diameter. After 1 month they became yellow in colour, as already noted in former experiments with beef broth agar. These colonies were made up of oval bacilli morphologically similar to the nitroso-bacterium.

Potato used as Culture medium.

I have inoculated potatoes from ammonia solutions showing oxidation, on several occasions. Profuse growth always occurred, the colour being usually yellow. On 10 occasions subcultures were made in ammonia solutions; oxidation occurred twice.

Cultivations of the Nitroso-bacterium at 37° C.

I have made a few experiments with cultures containing nitroso-bacteria at this temperature. Ammonia solutions were inoculated with earth; these showed oxidation in about a month. Ammonia solutions were then inoculated with strongly nitrifying solutions and incubated, but these failed to show any evidence of oxidation. It would seem therefore that 37° C. is not well suited to these bacteria.

Anaerobic Cultures.

Tubes subjected to this form of culture failed to give any evidence of oxidation of the ammonia contained in them.

Finally I would mention that the length of time during which I have been engaged in this work was due in the first place to the fact that the functional power of the nitroso-bacterium is only displayed when the micro-organism is in considerable quantity, and that it is easily lost. It has frequently occurred that cultures known to contain this species failed to show any oxidation of the ammonia in which they were inoculated. This happened in about 30 % of the solutions, and at least 100 plates. Secondly, the time required by the bacterium to oxidise ammonia was lengthy; two months was the average time, and there were several instances in which 4, 5, 6, 9, and even 12 months were required.

Summary of Results.

In the first place cultures of the nitroso-bacterium were developed in inorganic solutions. These carried to a 5th dilution exhibited practically a pure culture of this species.

Secondly, cultures of the nitroso-bacterium were inoculated into solutions containing small quantities of organic matter. In these they were able to oxidise the ammonia present. It was found that a culture developing in the presence of small quantities of organic matter was better able to oxidise the ammonia in higher percentages of organic matter than a culture taken directly from an inorganic solution.

In growing this species on plates silica jelly has the disadvantage of being difficult to prepare. It was 18 months before I obtained a satisfactory plate. This jelly grows the nitroso-bacterium well, but other species can also develop colonies on it. Ammonia agar also grows the species, as shown by the oxidation of the ammonia, and the colonies assume a characteristic form.

As to beef broth agar and gelatine, these media grew colonies of a micro-organism similar to the nitroso-bacterium from oxidised ammonia cultures. Pieces of these plates containing such colonies in pure culture frequently oxidised ammonia in solutions. On the other hand pieces of such plates showing no colonies never produced any oxidations of the ammonia. Furthermore the single colonies from silica and ammonia agar plates, which oxidised the ammonia in the media

into which they were sub-cultured, grew well on beef broth agar and gelatine.

From the above results I have come to the following conclusions:

(1) That the nitroso-bacterium grows well on any ordinary medium.

(2) That the supposed parallel organism is no other than the nitroso-bacterium itself.

(3) That in the presence of large percentages of organic matter the nitroso-bacterium, although growing very profusely, loses for a time the power of converting ammonia into a nitrite.

In conclusion I beg to offer my best thanks to Dr Blaxall, Bacteriologist to the Government Lymph Laboratories and Westminster Hospital Medical School. On his advice I undertook this work, and through his kindly help I was enabled to carry out the little that I have done in contributing to the study of the micro-organisms associated with the nitrifying process, whose life history is as yet but imperfectly known and whose functions are of such far-reaching importance.

A PIPETTE FOR DILUTING SERUM, ETC.

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An instrument which I had made several years ago presents some advantages and may therefore be of use to others who have to deal with serum-diagnosis, etc. The chief advantage of the instrument is that after each dilution has been made it is ready for making further dilutions without cleansing it. The instrument is moreover easy to manipulate. It may be described as essentially a "reversed Thoma-Zeiss pipette," the graduations being marked upon the upper section of the tube, instead of on the lower section. The accompanying figure makes the construction of the instrument clear.

The method of using the pipette is as follows: A rubber tube and mouth-piece having been affixed to the upper end, the pipette is filled with saline solution or other diluent up to the mark desired for the intended dilution. The pipette is now laid horizontally, and the fluid to be diluted (serum, etc.) is allowed to flow into the pointed end from a fine capillary, until the top graduation mark is reached. The contents are then blown out into a watch-glass. It will be noted that the lower section of the pipette is longer than the upper, or graduated section; the object of this is to prevent the entrance of the serum into the bulb. The expulsion of the fluid from the pipette, the small amount of serum being expelled first and being followed by a relatively large volume of diluent, secures a practical cleansing of the instrument. In order to prove that no appreciable amount

of serum remains in the instrument, after diluting samples of very potent
serum, the lower section of the instrument was filled with fresh saline
solution, and these were found to possess no agglutinating properties.
Another point is that the serum might diffuse up into the bulb during
the manipulations; this does not take place however within a reasonable
time, as may be proved by staining the serum, thus enabling one to de-
termine the limits of its diffusion. From the fact that a certain amount of
the diluent remains within the pipette, when used in the above-described
manner, there is naturally a slight error. In order to determine the
extent of this error, the pipettes were weighed dry, full of distilled
water, and again after expulsion of the water. Tested in this way,
the mean of three weighings gave an error of 1 in 162, a negli-
gible quantity in practical work. If it is desired to avoid this small
error, the diluted serum may be sucked up into the instrument and
again expelled, but this would entail cleansing the pipette for further
use. In practice the error above noted needs scarcely to be taken into
account.

Three sizes of pipette were made for me, as follows :—

a gives dilutions from 1:100 to 1:1000, its capacity being about 570 mg. of water.
b   „   „   „   1:50 to 1:500,   „   „   „   „   370 mg. „ „
c   „   „   „   1:10 to 1:100,   „   „   „   „   430 mg. „ „

The fact that the pipettes are made by Zeiss (Jena), may be taken
as a sufficient guarantee for the accuracy of the graduations, and of the
neatness of finish.

Where it is desired to make an extensive series of serial dilutions, I
have employed the above-described instrument in conjunction with the
method which I published in the *Edinburgh Medical Journal* for 1897 ;
see also R. C. Cabot (1901), *A Guide to the Clinical Examination of the
Blood for Diagnostic Purposes* (New York : William Wood and Company).
In this latter method the possession of a number of calibrated capil-
laries, ranging from 5 to 100 c.mm. capacity, greatly increases the value
of the instrument.

THE RELATION OF SULPHUR IN LIGHTING-GAS TO AIR VITIATION.

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(*From the Physiological Laboratory, Oxford.*)

IT is matter of common observation that air which is much vitiated by combustion of lighting-gas is distinctly oppressive, apart altogether from the rise of temperature which is always associated with the vitiation. This effect is always very evident if the proportion of CO_2 in the air of a room has been raised to 30 or 40 volumes per 10,000 by combustion of gas. Air to which pure carbonic acid has been added in the same proportion has no such effect, however, and is practically indistinguishable from pure air. Deficiency of oxygen to such an extent as occurs in the air of a room is also without sensible effect. In coal-mines, where the air is commonly vitiated to a considerable extent by slow oxidation in the strata, it is, for instance, quite impossible to distinguish by the senses pure air from air containing an excess of 50 or even 100 volumes per 10,000 of CO_2, with a correspondingly large deficiency of oxygen. The unpleasantness of air vitiated by combustion of lighting-gas is therefore not due to excess of carbonic or deficiency of oxygen.

In a recent paper in this Journal[1] I remarked that the sulphur present in gas is probably the cause of the unpleasantness. The quantity of sulphur in ordinary lighting-gas is, however, so small that this hypothesis may at first sight seem improbable. As a rule English gas contains less than 20 grains (1·3 grammes) of sulphur per 100 cubic feet, or 0·46 grammes per cubic metre. Hence, as gas forms in burning about half its volume of CO_2, the products of combustion would contain

[1] Vol. II. p. 424, 1902.

less than 0·5 grammes of sulphur to 500 litres of CO_2. If the sulphur were oxidised to SO_2, this would correspond to about one gramme or 0·33 litres of the latter to 500 litres of CO_2, or one volume of SO_2 to 1500 of CO_2. Hence air vitiated by combustion of gas to the extent of 30 volumes of CO_2 per 10,000, would usually contain less than $\frac{30}{1500} = 0·02$ volumes of SO_2 per 10,000 of air, or about one part in 500,000. This is a very small proportion; but not very much less than what Lehmann[1] found to produce perceptible irritation of the nose and throat. He showed that this effect occurred with air containing about one part in 200,000 of air. He records no experiments with smaller proportions.

There is no doubt, however, that the sulphur is partly, at least, present as sulphuric acid and in a particulate form. My attention was first drawn to this fact by an observation related to me by the manager of a weaving shed in a small country town. He informed me that occasionally a fog had occurred in the shed when the gas was lit, and that at the same time the air became most unpleasant and irritating. This was clearly due to the purification of the gas being occasionally worse than usual, so that a considerable proportion of H_2S passed through the purifiers. The quantity of sulphuric acid formed was thus so great that although the ventilation was especially good the air of the shed became foggy from the condensation of moisture on minute drops of sulphuric acid in the air. As will be shown below, it is easy to produce evident fog in a room by burning gas containing an extra proportion of sulphur. Sulphuric acid suspended in a particulate form has not the same specific taste as sulphurous acid, but is extremely irritating and unpleasant.

The sulphur contained in crude unpurified lighting-gas is present chiefly as sulphuretted hydrogen, about a tenth being present in other forms, chiefly as carbon bisulphide. In the process of purification the H_2S is easily removed by quicklime, hydrated oxide of iron, or manganese dioxide ("Weldon mud"). The sulphur in other forms amounts usually to about 30 to 40 grains (2 to 2·7 grammes) per 100 cubic feet (2·8 cubic metres) of gas. When, as is usually the case in England and America, the CS_2 is also removed, this is effected by passing the gas through a purifier charged with sulphide of lime, which has the property of absorbing CS_2. The purified gas contains only about 0·5 gramme (6 or 8 grains) of sulphur per 100 cubic feet when the sulphide purifier is working perfectly. If, as is sometimes the case even in large towns, sulphide of lime purification is not used, the gas which is distributed

[1] Archiv für Hygiene, XVIII. p. 180.

may contain 2 to 2·7 grammes (30 to 40 grains) of sulphur per 100 cubic feet. The averages of the daily official tests of the gas of the three London companies for 1902 were 11·1, 12·2, and 9·2 grains of sulphur per 100 cubic feet.

The large gas companies in England are usually legally bound to supply gas containing less than a certain maximum amount (about 20 grains) of sulphur per 100 cubic feet. For London the maximum is at present 17 grains in summer and 22 grains in winter. The importance of keeping the proportion of sulphur in gas as low as possible is thus very generally recognised, although less attention has been given to the physiological action of the products of combustion of sulphur than to their destructive action on the bindings of books and on other fabrics. In many of the smaller English towns, and certain of the larger ones, including Birmingham, gas containing as much as 30 grains of sulphur per 100 cubic feet is, however, regularly distributed.

In order to investigate the relation between the proportion of sulphur in lighting-gas and the unpleasantness of air vitiated by the products of combustion I employed the following method. In one of two rooms the ordinary Oxford gas (which contained about 8 or 9 grains of sulphur per 100 cubic feet) was burnt. In the other room gas was used to which any required proportion of CS_2 vapour had been added. The carbonic acid was determined at intervals in the two rooms, so that the degree of admixture of the products of combustion with the air was known.

The CS_2 vapour was added to the gas by the following method. By means of a three-way junction the stream of gas passing to the burner was divided into two portions, one of which passed over liquid CS_2 contained in a wide test-tube. The relative quantities of gas passing in each portion were regulated by means of taps. The two streams were afterwards re-united by a second three-way junction, and then proceeded to the burner. By regulating the taps the proportion of CS_2 vapour in the united stream could be regulated at will. The stream passing over the carbon disulphide was relatively very small, and in order to facilitate its regulation the gas was allowed to bubble through about half-an-inch of water in a test-tube before passing to the CS_2. By counting the bubbles it was easy to regulate the stream to about the proper amount, and any variation could be detected at once.

The sulphur in the gas was estimated by Harcourt's method[1]. This method depends on the fact that in presence of hot platinised pumice

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[1] Fully described in Butterfield's *Gas Manufacture*, p. 218.

CS$_2$ in gas is broken up with formation of H$_2$S, which is estimated by passing the gas in a slow stream of small bubbles through lead acetate solution, until the colour becomes equal to that in a standard tube containing a perfectly stable brown solution. As a small portion (about 6 or 8 grains of S per 100 cubic feet of gas) of the sulphur in gas is not affected by this platinised pumice, it is necessary to add to the result obtained about 6 to 8 grains (0·5 gramme) in order to get a true result. The small stream of gas required for the test was taken off just before the mixed stream of gas reached the burner. Frequent determinations were made during the experiments. The ordinary Oxford gas was found to show only from 1 to 2 grains of S by Harcourt's test, and was therefore taken to contain 8 to 9 grains in all. The CO$_2$ in the air was determined by the rapid method which I described in this Journal, Vol. I. p. 109, and Vol. II. p. 415.

The effects produced by the air in the two rooms, with varying percentages of CO$_2$ in the air and of S in the gas burnt, were observed at intervals by myself and others, and noted at the time. The impure gas was burnt in Room 1, and the ordinary Oxford gas in Room 2. Several experiments were made on different days. The burners employed were the ordinary ones (Bray's fishtail burners) used in the two rooms.

The following are the notes of an experiment.

Gas lit at 12.45 p.m. Stream of gas passing over CS$_2$ in Room 1 adjusted to 32 bubbles per minute. Sulphur = 34 grains per 100 cubic feet.

1.15 p.m. Room 1. CO$_2$ = 18·5 volumes per 10,000. 30 bubbles per minute. Air markedly unpleasant, causing perceptible irritation of the air-passages, and having a slight taste of sulphurous acid.

1.30 p.m. Room 2. CO$_2$ = 20·5 volumes. Air not appreciably unpleasant.

1.45 p.m. Room 1. CO$_2$ = 25 volumes. 38 bubbles per minute. Sulphur = 46 grains per 100 cubic feet. Air very unpleasant. Distinct irritation of air-passages, and slight irritation of the eyes. Acid taste.

2.50 p.m. Room 1. CO$_2$ = 20 volumes. Sulphur = 41 grains per 100 cubic feet. Air irritating and unpleasant. Temperature 20° C.

3.5 p.m. Room 2. CO$_2$ = 36 volumes. Air slightly unpleasant, but much less so than in Room 1. Temperature 20° C. No acid taste perceptible.

4.0 p.m. Sulphur in ordinary gas = 8·7 grains per 100 cubic feet.

The same experiment was repeated on several days, with slight variations. With 40 grains of sulphur per 100 cubic feet of gas a taste of sulphurous acid and slight irritation of the air-passages were distinctly noticed at about 13 volumes of CO$_2$ per 10,000. With only 9 or 10

grains of sulphur, on the other hand, similar irritation of the air-passages was not noticed until the CO_2 rose to about 35 or 40 volumes; and even at this point no acid taste could be detected—possibly because any sulphurous acid formed had been almost entirely oxidised to sulphuric acid. It was thus evident that the unpleasantness of the air varied in proportion to the amount of sulphuric and sulphurous acid present.

With 51 grains of sulphur in the gas, and 39 volumes of CO_2 in the air, distinct fog was observed in the room, although no moisture had condensed on the windows, and the air was only 81 % saturated with moisture, as shown by the readings of dry and wet bulb thermometers (23·5° and 21·5°). The air was extremely unpleasant. The fog was more evident when with the same gas burning and the air 82 % saturated with moisture, the CO_2 had risen to 48 volumes. When the air contained about the same proportions of moisture and CO_2, with only the ordinary gas burning, no distinct fog could be seen.

Several other individuals besides myself compared the air in the two rooms, and all agreed that the air was far more unpleasant in Room No. 1 than in Room No. 2. They easily perceived the acid taste and unpleasantness of the air in Room No. 1. In one individual, sneezing was produced on going into Room 1 with 35 volumes of CO_2 in the air, and 36 grains of sulphur in the gas. Two others complained of slight headache after a few minutes. From the results as a whole there could be no doubt whatever as to the great superiority from the standpoint of comfort and health, of gas properly purified by sulphide over gas from which the carbon bisulphide is not removed.

Unfortunately no process is known by which the whole of the sulphur can be removed from lighting-gas. Were it possible to remove the whole there would apparently be no objection whatever to using gas perfectly freely for both heating and illuminating purposes in any ordinary room, and allowing the products of combustion to escape into the room. With a clean paraffin lamp, burning good oil, I found that the air of a room was not noticeably unpleasant, apart from the heat, even when the CO_2 had risen to 75 volumes per 10,000. With the Oxford gas, on the other hand, the air was distinctly unpleasant when vitiated to the extent of 30 to 40 volumes of CO_2, and very unpleasant when vitiated to 60 volumes. The small quantity (8 or 9 grains per 100 cubic feet) of sulphur in the gas was evidently responsible for the difference.

In judging of the hygienic importance of varying proportions of

sulphur in lighting-gas it is of course necessary to take into consideration the illuminating power of the gas. To take an example, the gas now distributed in London south of the River has with ordinary burners only about half the illuminating power of the gas distributed in several towns in Scotland, and about two-thirds of the illuminating power of Liverpool gas. To obtain the same light with a good flat-flame burner it is thus necessary to burn about 100 % more gas in London south of the River than in the Scotch towns, and 50 % more than in Liverpool. A given percentage of sulphur is therefore correspondingly more important with the poorer than the richer gas if flat-flame burners are used. With Welsbach burners the difference is much less important.

CHIEF CONCLUSIONS.

1. The unpleasantness of air vitiated by the products of combustion of lighting-gas is due to the presence of sulphur in the gas, and varies in proportion to the amount of sulphur.

2. Gas which is purified from carbon bi-sulphide is greatly superior from the hygienic standpoint to gas which is only purified from sulphuretted hydrogen.

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THE PATHOGENICITY OF B. COLI IN RELATION TO THE BACTERIOLOGICAL EXAMINATION OF WATER.

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In this paper the term *B. coli* is used in its most restricted sense, and as implying organisms having all the characters of the typical *B. coli communis*. It is a well-ascertained fact that *B. coli*, even when the term is so restricted, exhibit very varying virulence.

From the point of view of the bacteriological examination of water the question of the virulence of isolated *B. coli* has been raised by some workers to a position of much importance, and the pathogenicity of such bacilli, or of the incubated broth and water, has been advocated as the best criterion of the purity of a drinking-water.

The matter is one of considerable practical importance since it must be admitted that true *B. coli* in water may be derived from quite different sources and so possess different significance. If we could accurately determine their source, or at least divide into two groups those from harmful sources and those from harmless, a great step in the bacteriological examination of water would be attained and it might be possible, and with great confidence, to pass *B. coli* in large numbers in water from one source, while condemning a water from another source with perhaps less *B. coli* but of harmful origin.

It might be thought that the virulence of the isolated *B. coli* would be of service as such an indicator of the source, harmful or harmless. In considering this question I have based my conclusions and deductions in part upon the recorded results of other workers, and in part upon experiments of my own.

Levy and Bruns[1] have strongly advocated the importance of the

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[1] *Archiv f. Hygiene*, Vol. xxxvi.

virulence test. Their view is, that if genuine *B. coli* can be demonstrated in water it is a proof of the *faecal contamination* of that water.

Such genuine *B. coli* can be distinguished, according to these authors, from what they call the coli-form varieties which occur in quite unobjectionable supplies, by testing their virulence.

They assert that 0·5 to 1·0 c.c. of a 48 hours' broth culture of *B. coli* derived from normal human faeces will if intraperitoneally injected into a guinea-pig kill the animal in 1—3 days, while in diseased conditions a much higher virulence may be exhibited. They further state that virulent coli races gaining access to water will subsist there and maintain their virulence for several weeks, while they have never come across a coli-form isolated from water capable of killing a guinea-pig in doses of 1—2 c.c. when injected intraperitoneally. The method they suggest is to treat 100 c.c. of the water with 1% peptone and 1·5% common salt and incubate at 37° C. for 48 hours. Guinea-pigs are then inoculated with 1—2 c.c. intraperitoneally; mice with 0·2 to 0·5 c.c. subcutaneously; rabbits with 2—3 c.c. intravenously.

With contaminated waters the animals will die, and virulent *B. coli* will be found at autopsy, with or without other species.

Blachstein[1] advocated a similar method, *i.e.* the injection of water after incubation with broth. His paper is however very inconclusive.

Weissenfeld[2] has also investigated this question. He examined 56 good and bad waters. His animal experiments were made either with pure broth cultures of the isolated organism, or, as done by Levy and Brun, and also by Blachstein, with mixed cultures of broth and water. His dose was 1 c.c. of a two days' old culture injected intraperitoneally into a guinea-pig of medium weight.

He found that from the results of the animal experiments no general rule could be enunciated. *B. coli* from, so-called, good waters were pathogenic or non-pathogenic, while with his bad waters both classes were also obtained. He concludes that the isolation of a virulent *B. coli* does not, of necessity, indicate faecal contamination.

His points of identification for his *B. coli* are however very inadequate. The characters he takes are :—vine-leaf surface gelatine colonies; gas in sugar agar stab; bacilli more or less motile, often not motile, decolorised by Gram. He states that he attaches no importance to milk-souring and indol production, while he does not mention

[1] *Annales de l'Institut Pasteur*, 1893, VII. p. 689.
[2] *Zeitschr. f. Hygiene*, 1900, XXXV. p. 78.

acid production or fermentation with different sugars, apart from gas in sugar agar stab cultures. He also classifies his waters into good or bad, but from the details appended this is rather an arbitrary classification. Compared with my results, given below, he obtained a high percentage of virulent *B. coli*. No mention is made, or I have overlooked it, of autopsies on the killed animals and recovery of the inoculated bacillus. He apparently also only examined 1 c.c. and 1 litre of the water.

In considering the question of virulence of *B. coli* in water supplies it must be remembered that it has no, or but very slight practical importance from the point of view of the possible *direct harmfulness* of such a virulent bacillus. *B. coli* are looked for in water not because they themselves are harmful, actually or potentially, but because they are *indicators* of contamination. In this sense they may be compared to the estimation of, say, chlorine in chemical water analysis, and the question of their direct harmfulness is no more at issue than that of the direct harmfulness of chlorides in water.

Also *B. coli* are not present in *perfectly pure* water and therefore their presence must be looked upon as indicating contamination, but by no means contamination of necessity dangerous. The question of virulent *B. coli* can therefore be narrowed down to the following :—Does the fact that an isolated *B. coli* is pathogenic, when obtained from a water, indicate that the contamination is harmful and of necessity dangerous, and does the fact of its being non-virulent indicate freedom from dangerous pollution? Such a conclusion can be by no means maintained. Sewage and human faecal contamination are the two chief dangers to water supplies and constitute dangerous contamination. For a virulent *B. coli* to be a true indicator of such dangerous contamination it is obvious two conditions must be fulfilled. In the first place *B. coli* from such sources must be virulent, or at least the majority must be virulent, and secondly such virulent *B. coli* must be able to maintain their virulence for at least several weeks after obtaining access to a water supply.

Levy and Brun state that both these conditions obtain, but this is not confirmed by other workers. Thus Lartigau[1] states "general experience abundantly demonstrates that the bacillus (*i.e. B. coli*) is on the whole non-pathogenic as ordinarily found in normal faeces." Lartigau quotes a number of authors whose results confirm this opinion.

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[1] *Journ. American Med. Assoc.* April 12th, 1902.

Klecki[1] found the virulence of *B. coli* from the intestinal contents of a normal dog to be in general very variable. Harris[2] found a number of *B. coli* isolated from human faeces and from sewage to be non-virulent to guinea-pigs and rabbits. On the other hand this worker found *B. coli* from abnormal conditions of the intestine to be virulent. Lartigau (*ibid.*) states that alterations from the conditions normally present in the gut soon increase the virulence of the contained *B. coli*, while Sanarelli has shown that the virulence of *B. coli* in the intestines is increased in cases of enteric fever.

On the whole it seems probable that *B. coli* from human faeces and from sewage are in general of relatively low virulence. If that is so the fact that an isolated *B. coli* from water is non-virulent cannot in any way be taken as an indication that it is from a source which is harmless and can be neglected.

Again, the second condition, that virulent *B. coli* will maintain their virulence in water for some time is doubtful, and some experiments recorded below negative it.

There is however another aspect to the problem. It is generally recognised that in inflamed and abnormal conditions of the gut (including enteric fever) the virulence of intestinal *B. coli* is greatly increased. If therefore a virulent *B. coli* is found in a water supply, may it not be an indication of contamination from such sources, and also of fairly *recent* contamination, for it is probable that *B. coli* will lose their virulence gradually in water?

This may possibly be so, but to be able to definitely affirm it we must have a considerably greater knowledge of the virulence of *B. coli* from comparatively harmless sources such as sheep-dung, than we at present possess.

I will now consider the results of my experiments. In all work dealing with *B. coli*, owing to the different interpretations of this term, it is necessary to give the cultural characters of the organisms isolated. These are given in Table I. (p. 392). In Table I *a.* (p. 394) are given the characters of four doubtful or allied organisms which were also examined. It will, I think, be admitted that all organisms included in Table I. are certainly true *B. coli*.

Table I. includes a record of bacteria from 22 different sources. Of these 15 are from water (2 being from sea water), 2 from milk,

[1] *Annales de l'Institut Pasteur*, 1895, IX. p. 710.
[2] *Journ. of Pathology and Bacteriology*, VII. No. 1.

TABLE I. *Cultural characters of the B. coli inoculated.*

No.	Source	Morphology and Motility	Gelatine surface colonies	Gelatine slope	Broth	Litmus milk Acid	Litmus milk Coagulation	Potato	Production of gas Lactose media	Production of gas Glucose media	Neutral red reaction Glucose agar shakes	Indol
1	Milk	Short thick bacilli. Sluggish motility	—	Translucent growth. No liquefaction	Uniform turbidity. No scum	+	+ 6 days	Whitish growth	+	+	Not examined	−
2	Milk	,, ,,	—	,,	,,	+	+ 24 hrs.	Yellow brown growth	+	+	+	+
3	A deep well water	Short thick bacilli. Actively motile	—	,,	,,	+	+ 9 days	Yellow growth	+	+	Not examined	+
4	Valves of heart. Case of malignant endocarditis	,, ,,	—	,,	,,	+	+ 7 days	Pale yellow growth	+	+	+	+
5	A pure uncontaminated upland surface stream	Short thick bacilli. No true motility		,,	,,	+	+ 6 days	,,	+	+	+	+
6	Upland surface reservoir. A pure supply	Short thick bacilli. Sluggish motility		,,	,,	+	+ 2 days		+	+	+	+ (traces)
7	Typhoid excreta	Short bacilli. No true motility		,,	,,	+	+ 3 days	Yellow brown growth	+	+	+	+
8	,, ,,	Short bacilli. Sluggish motility		,,	Uniform turbidity. Scum	+	+ 2 days	,,	+	+	+	+
9	Upland surface reservoir. Contaminated	Short bacilli. No true motility	Quite typical	,,	Uniform turbidity. No scum	+	+ 3 days		+	+	+	+
10	A deep well water. Contaminated	Short bacilli. Motile	,,	,,	Uniform turbidity. Scum	+	+ 3 days	Yellow brown growth	+	+	+	+

	Source	Morphology	Typical			Turbidity	+	Growth	Colour	+	+	+	+
11	Spring and upland surface water. Markedly contaminated	Short bacilli. Sluggishly motile	"	"	"	"	+	+ 3 days	Pale yellow growth	+	+	+	+
12	The same source as No. 11	" "	Not quite typical	"	"	"	+	+ 3 days	White growth	+	+	+	+
13	A contaminated well water	Very short bacilli. Sluggishly motile	Quite typical	"	"	"	+	+ 2 days	White growth	+	+	+	+
14	Sewage	Short bacilli. Fair motility	"	"	"	Uniform turbidity. No scum	+	+ 4 days	Yellow brown growth	+	+	+	+
15	Human excreta "enteritis"	Short bacilli. No true motility	"	"	"	"	+	+ 24 hrs.	Colourless growth	+	+	+	+
16	Upland surface water	Short bacilli. Sluggish motility	"	"	"	Uniform turbidity. Scum	+	+ 3 days	White growth	+	+	+	+
17	A contaminated well water	" "	Atypical	"	"	Uniform turbidity. No scum	+	+ 2 days	Colourless growth	+	+	+	+
18	A deep well water. Contaminated	Short bacilli. No true motility	Quite typical	"	"	"	+	+ 2 days		+	+	+	+
19	Sea water. Slightly sewage contaminated	" "	Atypical	"	"	"	+	+ 4 days	Pale yellow growth	+	+	+	+
20	Sea water. Markedly contaminated	Short bacilli. Motile	Quite typical	"	"	"	+	+ 4 days	"	+	+	+	+
21	A pure mixed spring and upland surface water	Short bacilli. Sluggishly motile	"	"	"	Uniform turbidity. Thin scum	+	+ 4 days	"	+	+	+	+
22	A contaminated well water	Short bacilli. No true motility	"	"	"	"	+	+ 4 days		+	+	+	+

TABLE I a.   *Organisms allied to B. coli in many of their characters.*

No.	Source	Morphology and Motility	Gelatine surface colonies	Gelatine slope	Broth	Litmus milk		Potato	Gas production		Neutral red reaction Glucose agar shake	Indol (10 days' peptone water)
						Acid	Coagulation		Lactose	Glucose		
a	A mountain stream, uncontaminated except from sheep excreta	Short bacilli. No true motility*		White semi-translucent growth. No liquefaction	Uniform turbidity. No scum	Alkali produced	No coagulation	Yellow-white growth	–	+	+	+ (traces)
b	A pure spring water	Short bacilli. No true motility*		,, ,,	Uniform turbidity. Thin scum	,,	,,	Pale brown growth	–	+	Partial	+ (slight)
c	A pure upland surface water (from reservoir)	Very short bacilli. Showing distinct motility	Typical	,, ,,	Uniform turbidity. No scum	+ Acid produced†	,,	Pale yellow growth	–	+	+ (complete in 48 hrs.)	traces only
d	From body of oyster	Short bacilli. Very sluggish motility	Atypical	,, ,,	,, ,,	Alkali produced‡	,,	Abundant yellow growth	–	+	+	+ (marked)

* After being kept in the Laboratory for some time they both showed sluggish motility.

+ The milk tubes became acid in 24 hrs. and did not subsequently become alkaline (kept for one month).

‡ Some preliminary acid production, distinctly alkaline after one week.

3 from excreta, and 1 each from sewage and a case of malignant endocarditis.

The inoculations were made mainly into guinea-pigs and mice, the results being given in Table II. It will be noticed that in most cases the dose inoculated was very large both for guinea-pigs and mice. To ensure the maximum effect the inoculation was made intraperitoneally. Table II. shows that even when these massive doses were inoculated intraperitoneally into guinea-pigs the results were negative for most of the injected organisms. The guinea-pigs used were of approximately equal weight (270–320 g.).

The results may be further classified as follows :—

Source	Virulent to guinea-pigs	Non-virulent to guinea-pigs
Pure water	1	2
Suspicious water	0	3
Contaminated water	3	6
Sewage or excreta	0	3
Valves of heart (Malignant Endocarditis)	0	1
	4	15

The figures are not large but, as far as they go, they show that $\frac{1}{3}$ of the B. coli from both pure and contaminated sources were virulent, while it is significant that all 3 organisms from excreta or sewage were non-virulent.

It certainly was not true for these waters that a virulent B. coli indicated a bad water and a non-virulent a good water.

It should be added that the distinction between pure, suspicious, and contaminated waters was based not upon a single examination, but upon an intimate knowledge of the waters in question, both from the point of view of their liability to pollution and from the figures of bacteriological and chemical analyses made every three months for at least $2\frac{1}{2}$ years. It is not thought necessary to give particulars of these waters.

It is of interest to note that the water supply from which bacteria 12 and 13 were obtained should on the only two occasions on which the virulence was tested, have yielded B. coli both of which were distinctly pathogenic. This water showed marked evidence of contamination. Thus the sample from which No. 11 was isolated contained 330 and 2600 organisms per c.c. growing on agar (37° C.) and gelatine (22° C.) plates respectively, while B. coli was readily isolated from 0·5 c.c. of the water.

TABLE II. *Inoculation experiments.*

No. of organism	Source	Character of source	Guinea-pig inoculations			Mouse inoculations		
			Dose	Method of inoculation	Result	Dose	Method of inoculation	Result
3	A deep well water	A suspicious water	1·5 c.c. 24 hrs. broth culture	Intra-peritoneal	No effect			
5	Upland surface stream	Pure uncontaminated water	2 c.c. 3 days broth culture	„	„			
6	Upland surface water	„	„ „	„	„			
9	„ „	A contaminated water	Standard dose*	„	„	1 c.c. whey from coagulated milk 4 days old	Intra-peritoneal	Death in about 24 hrs.
10	A deep well water	„	„ „	„	„	„ „	„	Death in about 30–40 hrs.
11	Mixed spring and upland surface water, in reservoir	Markedly contaminated	„ „ A second guinea-pig inoculated with same dose	„ „	Dead in less than 20 hrs. Dead in about 50 hrs.	„ „	„	Death in less than 16 hrs.
12	Same source as 11, but examined three months later and isolated from a fresh sample	„	Standard dose	„	Dead in about 48 hrs.			
13	A well water	„	„ „	„	No effect	„	„	Dead in 20hrs., repeated many times with the same result
16	Upland surface water	A suspicious water	„ „	„	„			
17	A well water	„	„ „	„	„			

No.	Source	Character	Dose		Effect	1 c.c. old broth culture		Death in less than 20 hrs.
18	Deep well water	Contaminated	"	"	"	1 c.c. old broth culture	"	Death in less than 20 hrs.
19	Sea water	Contaminated with sewage, but not markedly	"	"	Dead in about 40 hrs.			
20	" "	Markedly sewage contaminated	"	"	No effect			
21	Mixed spring and upland surface water	A pure water	"	"	Dead in about 22 hrs.			
22	Well water	Contaminated	"	"	Local tissue necrosis. Recovery			
4	Malignant endocarditis (valves)	—	1·5 c.c. broth culture (3 days)	"	No effect			
7	Typhoid excreta	—	2 c.c. broth culture (3 days)	"	"			
8	" "	—	"	"	"	0·5 c.c. broth culture (3 days)	Subcutaneous	No effect
14	Sewage	—	Standard dose	"	No effect	" "	Intra-peritoneal	Dead in less than 20 hrs.
15	Human excreta, "enteritis"	—	"	"	"			
a	Upland surface water	Contaminated	2 c.c. of 1 week's broth culture	"	"			
b	A pure spring water	A pure water	Standard dose	"	"			
c	Upland surface water	A pure water	"	"	"	0·5 c.c. broth (1 week old)	"	
d	Oyster	—	"	"	Dead in about 50 hrs.			"

* *Standard dose* is made by adding the scrapings of an agar sloped culture 24 hours old to 5 c.c. of a 5–6 days old broth culture of the same organism. The whole enriched 5 c.c. is injected.

The pure water from which the virulent *B. coli* was isolated consists partly of spring and partly of upland surface water from the hill sides. It is collected directly into a reservoir.

It is a very pure water and it is rare to find *B. coli* even in as much as 50 c.c. In this particular sample *B. coli* was found in 40 c.c., but not in smaller amounts, while the numbers of organisms per c.c. were 3 at 37° C., and 145 at 22° C.

The results of inoculations on mice are of some interest.

Five *B. coli* from waters and one from sewage were all pathogenic to mice when injected intraperitoneally in large doses. Several mice, as controls, were injected with 1 c.c. of sterile milk intraperitoneally and showed no permanent ill effects, so the inoculation results must be ascribed to the organisms injected. In every case, mouse, guinea-pig, or rabbit, an autopsy was made on the animals killed by inoculation, and the *B. coli* recovered from the spleen. All the *B. coli* used seemed to have sufficient virulence to kill mice when injected intraperitoneally. For the two *B. coli* isolated from milk, rabbits were used for testing the virulence :—

No. 1 inoculated intraperitoneally in a dose of 5 c.c. of a 24 hours' broth culture, killed the animal in less than 24 hours, and the same bacillus was recovered from the internal organs.

No. 2, with an equal dose injected intraperitoneally, produced no effect.

A few experiments on altering the virulence of *B. coli* were performed. With bacillus No. 11 two parallel inoculations were made.

Exp. A. Sterile tap-water in a flask was inoculated with this organism and the flask then kept outside the laboratory, in the open air, for 12 days. Subcultures were then made on to an agar slope and into glucose broth. Both subcultures were grown for 5 days, then 3 c.c. of the broth culture plus the growth scraped from the agar slope were inoculated intraperitoneally into a guinea-pig. Animal ill for the first 24 hours but subsequently recovered completely.

Exp. B. (Control.) The organism was grown in glucose broth for 12 days at 37° C. Then subcultures were made on an agar slope and into glucose broth as in Exp. A. After 5 days' growth the same dose was inoculated into a guinea-pig of equal weight. Animal dead in less than 17 hours. Bacilli recovered from the spleen. Here a diminution of virulence resulted from 12 days' growth in water.

Bacillus No. 21 when first inoculated a few days after its isolation, the standard dose killed a guinea-pig (wt. 270 g.) in 22 hours. After being grown in sterile water (plus 2 drops of sterile broth) at room temperature for two weeks, 6 c.c. of a 5 days' old broth, plus scraping from an agar slope failed to kill and showed no effect upon a guinea-pig (wt. 450 g.). For this inoculation, unfortunately, an equal sized

guinea-pig was not available and the dose was not proportionately increased so the result is not conclusive, but as in the preceding experiment an apparent loss of virulence by growth in water is shown.

Several experiments were made to try and raise the virulence of these organisms. Thionot and Masselin[1] state " the virulence of this bacillus varies with different growths, but any growth may have its virulence greatly augmented by passing the bacillus by intrapleural injection through a series of guinea-pigs or rabbits."

It was not found possible to take *B. coli* non-virulent for guinea-pigs but virulent for mice, and make them virulent for guinea-pigs by passage through mice.

Thus *B. coli* No. 9 was passed through 3 mice by intraperitoneal injection, but then still failed to kill a guinea-pig.

No. 13 was passed through 5 mice by intraperitoneal injection. It was then capable of killing mice in doses of 0·5 c.c. by *subcutaneous* inoculation. After two further passages by subcutaneous inoculation it was still unable to kill a guinea-pig, even when the large 'standard dose' was used.

Conclusions.

These experiments lend no support to the view that the pathogenicity of isolated *B. coli* is of help in determining the potency for evil of the water examined. Virulence as a property of *B. coli* is, I believe, a very variable character and one which can be readily lost, and with greater difficulty acquired, and the view advanced by some writers, *e.g.* Harris (*ibid.*), that toxicity is a specific distinguishing character seems to be without foundation.

[1] Text-book, p. 272.

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BEYER, H. G. (1903) *Naval Hygiene.* Excerpt, bound in cloth, from " Reference Handbook of the Medical Sciences," pp. 154—184, 1 plate and 11 figures, etc. 27 × 20cm.

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* The size of the books is given roundly in centimeters.—ED.

400a

EDMOND-ISIDORE-ÉTIENNE NOCARD

Born at Provins (Seine-et-Marne), 29 January, 1850.
Died at Saint-Maurice, France, 2 August, 1903.

CAISSON ILLNESS AND DIVER'S PALSY. AN EXPERIMENTAL STUDY.

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A CAISSON consists of a steel cylinder which is sunk in water, and out of which the water is kept by means of compressed air. The men in the caisson are thus able to work on the bed of the river or the sea.

The top of the caisson is provided with an air-lock, a chamber fitted with air-tight doors and cocks, whereby the air can be compressed or decompressed as the men enter or leave. A large cock is utilised for rapid decompression during the passage of material, and a small cock for the slower decompression of men. The men frequently take advantage of the large cock, and by breaking the rules get out of the air-lock quickly.

Ten metres of water roughly correspond to 1 atm.: thus for every 10 metres or 33 feet a pressure of $+15$ lbs. to the sq. inch or $+1$ atm. is required to keep the water out of a caisson or diving-bell. At a depth of 100 feet a man would be exposed to $+3$ atm., at 200 feet to $+6$ atm. In the case of a diver the conditions are the same. Compressed air is delivered through a tube to his helmet and escapes by a valve, by which means the water is kept out of his dress. The pressure of the air must always be just greater than that of the water.

The numerous accounts of caissonier's and diver's sickness which have been published show that the sickness never attacks the men while under pressure, but only after decompression. The account of the symptoms given by Pol and Watelle[1] in 1854 may be taken as

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[1] *Ann. d'hygiène publique et de méd. légale,* 1854. Cit. after Bert, *La Pression Barométrique,* p. 380.

typical. These authors had charge of 64 men who were employed in a caisson on the banks of the Loire at $+3\frac{1}{4}$ atm. The men were compressed in 15 minutes, worked for a 4 hr. shift, and were—according to the rules—decompressed in 30 minutes.

The physiological effects observed in compressed air were very slight—pains in the tympanic membrane (relieved by opening the Eustachian tube); slowing of respiration and diminution in thoracic expansion; slowing of the pulse; increase of urinary secretion; a feeling of resistance to movement owing to density of the atmosphere; inability to whisper, attributed to the resistance of the compressed air to the finer muscular movements of the tongue. On decompression these authors felt a lively sensation of cold and a certain degree of breathlessness; while the pulse accelerated. The cold is due to the expansion of the air.

Pathological effects. There were 47 men out of the 64 who stood the work more or less well; 25 were discharged owing to sickness; 2 died. The slighter attacks were 14, and the serious attacks 16 in number.

The accidents without exception occurred after decompression.

Types of Cases.

I.   Embarrassed respiration, pains in the limbs, loss of appetite and digestive troubles, black stools, loss of flesh.

II.   A dazed condition, muscular pains and cramps, feelings of numbness, vomiting of black material. One day this man lost consciousness soon after decompression. The pulse was full and frequent, the face congested, the respiration short and stertorous with mucous rales. There was complete loss of muscular power. The man was bled, purged and blistered and recovered in four hours.

III.   Severe muscular pains with cramps, the skin cold, respiration embarrassed, the pulse small and slow.

IV.   A comatose condition like to drunkenness, with indistinct speech, dilated pupils, accelerated respiration and rapid pulse. Diplopia, deafness and vertigo persisted.

V.   Severe pain in the limbs and chest, embarrassed respiration. The man was discharged. He returned to the work without permission. On decompression he suddenly fell unconscious and died in 15 minutes.

VI.   Great oppression, with dulness and bronchophony, a rapid pulse, cold skin, continual cough, and clonic contractions of the limbs. Better

after 5 hours of care. On another occasion this man became comatose, with dilated pupils, loss of muscular power and subdelirium. He was bled—the venous blood was arterial in colour. The man recovered and was discharged.

VII. Vision disturbed and double, hearing abolished, respiration frequent, pulse frequent and high tension. Bled, venous blood arterial in colour.

VIII. Pains in the head, vertigo, cramps.

IX. A powerful man, aged 40. Died immediately after decompression.

X. Muscular pains. *Cured by recompression.*

XI. Very severe muscular pains, persisting for many days.

XII. A few minutes after decompression the man appeared to be dead ; unconscious, livid face, dilated pupils, embarrassed respiration, indistinct trembling of the heart, no pulse, involuntary micturition, black vomit. Given hot baths and massage. Pulse returned in 30 minutes. Very severe muscular pains, blindness and deafness, with a wretched pulse during the night.

Next day the man is better but mentally confused. He recovered, but feeble vision and dilated pupils persisted.

Pol once suffered himself from acute pain in the left shoulder or arm, with shivering and vomiting. It seemed to him that *emphysema* existed in these places. E. H. Snell likewise thought he could detect emphysema in one of the cases of joint and muscular pain ('bends' or 'la pressure') at the Blackwall tunnel; while Bucquoy records that a cupping-glass, applied by a skilled assistant, would not hold on to a painful knee-joint owing to the gas set free in the subcutaneous tissues.

Among the numerous other reports collected together by Paul Bert[1] and recently added to by E. H. Snell[2] we will quote some cases recorded by Babington and Cuthbert at Londonderry. The pressure reached 30—43 lbs.

As in all caisson works the men suffered joint and muscular pains or from 'bends.'

Cases.

I. The man suddenly fell unconscious on decompression after 4 hours at 28 lbs. He was cold and livid. There was right facial paralysis

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[1] *La Pression Barométrique.* Paris, 1878.

[2] *Compressed Air Illness.* London, 1896.

and strabismus : pupils immobile.   Pulse 150, small and irregular ; heart sounds almost inaudible: respirations 24—44, very irregular.   Bled— blood very black.   Died in 24 hours.

II.   Similar to I. but no facial paralysis.

III.   Sharp shooting pains in legs and thighs ; unable to walk, feet cold and numb ; legs anaesthetic ; was found with feet almost in the fire, and toes badly burnt.   Recovered in two days.

IV.   Very similar to III.   Recovered in a few days.

V.   Fell helpless and semi-comatose during decompression.   Could be roused to answer questions.   Coma passed off in 18 hours.   Totally paralysed from 4th rib, retention of urine.   Died 160 days after.

VI.   Similar to V. but paralysed from 8th dorsal vertebra.   Died after 30 days.

In sinking the foundations of a bridge at St Louis on the Mississippi a high pressure of + 50 lbs. was used.   The number of workers was 600, of whom 14 died and 119 were more or less affected[1].   On leaving the caisson the workers are stated to have been pale and fatigued.   Involuntary contractions and nose bleeding occurred in some.

As the depth increased the illnesses became more numerous and severe.   The men were not taken sick while in the caissons, but a few minutes to one hour after decompression.   At the greatest depth the shifts were reduced from 4 to 1 hour, and the engineer Eads states that this reduced the serious accidents to *nil*.   Visitors who stayed but a few minutes, and the workers of the locks, who were quickly compressed and decompressed, never suffered.   53 cases suffered from paralysis of the legs with, usually, epigastric pain.   Nearly all these recovered in from 1 day to 1 week.   The sudden deaths were preceded by coma, stertorous breathing and muscular spasms.

At Brooklyn Bridge 110 similar cases of illness occurred, with 3 deaths, as reported by Dr Andrew Smith[2].   J. Hunter[3] stated that at the Forth Bridge works " the joint pain is of all symptoms the most constant, and almost invariably it attacks the knee alone, or with other joints, rendering its poor victims from its severity absolutely helpless.... Another prominent symptom met with was epigastric pain accompanied by vomiting."

The following interesting case is reported by Dr Twyman in the *Brit. Med. Journal*, Vol. i. 1888, p. 190.   A man worked for 3 hours at

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[1] Cit. after E. H. Snell.   *Compressed Air Illness.*   1896.

[2] *Effects of Compressed Air*, Detroit, 1886.

[3] *Edinburgh thesis*, cit. after Snell.

+ 60 lbs. He was decompressed in 3 minutes. On the way home he was seized with severe pain in the right elbow joint, a little later his right knee gave under him and he fell and became semi-conscious. Acute pain in both elbows and knees followed and 6 hours after decompression he was found cold and almost pulseless. He recovered but spat blood for 3—4 days. Necrosis of the right femur followed and the leg had to be amputated. The cause of this was, no doubt, embolism of the medullary artery of the femur.

The following account given by one of the caissoniers in the Blackwall tunnel gives a graphic picture of the less grave form of accidents.

"What did it feel like to go in? Oh, just the same as anywhere else. You felt a wee bit giddy when you went in, and that was all.

"We stayed in for eight hours at a shift. We had half-an-hour for dinner, but some of the men would not come out for it. They took it inside with them. Coming out again it was not so bad, but just chilly—bitter chilly, cold as charity. The pains would come on afterwards, in an hour or so, or when you got into bed. Bends in the back, and the wrists, and the legs—just awful. Men would turn out in the middle of the night, and come back to the works, and get into the compressed air again, in the medical locks. They had a full dose of it for a start, and let the pressure drop gradually. Then they went back home to bed. Do them any good? Eh, mon, it's no for me to say. They said so, but I thought it was only humbug, a faith dodge. When I had bends I just jumped about and took a drap of guid whiskey—better than all your doctor's concoctions.

"I never felt happier than when I was in the compressed air. Always happy, and on the cheery side. Why, laddie, I would get up in the morning feeling very dour and queer, and just go into the workings, and then whistle and sing all day long. Not that you could hear the whistling, at least a man with my lungs, when the pressure was over twenty-five.

"The worst thing that could happen was for the electric light to fail. Then they burnt candles, and the mixture of smoke with the air gave them 'bends' of an extra special vigour." (He was in the No. 3 caisson, where the pressure was as high as 37 lbs. to the inch. The effect of that abnormally condensed atmosphere was to cause an overpowering sleepiness.) "You nodded, and didn't care if you went to sleep for ever, though it was all very nice and dreamy. When I was alone in that 'casoon' I had to rope myself up, lest I should fall

asleep and tumble to the bottom, 60 ft. below. It was better under the river than in the casoons, because under the river the air could escape into the Thames. Tobacco had no sting. Even Irish roll had lost its savour. The only stuff that had any flavour was four-ale. You weren't allowed to take it in, but you did. But you had to take the cork out first. If you didn't the bottle would burst. The finest men in the tunnel were the first to be knocked out. The men of delicate appearance stood the pressure best."

The symptoms of the men employed at Blackwall have been fully reported by E. H. Snell (*loc. cit.*). Snell especially brought into notice cases of auditory vertigo.

In none of these cases the vertigo occurred without deafness. The vertigo was increased by moving the head in one particular direction and was frequently accompanied by vomiting and nystagmus.

That very prolonged exposure to compressed air is harmless is shown by the fact that mules were kept in the Hudson caissons (at $+2\cdot3$ atm.) for many months, and were sold for a good figure at the end (E. W. Moir[1]).

Von Schrötter[2] has studied many caisson cases at Vienna where the depth of the water was 25 metres. In one case a strong, healthy man aged 36 worked at $+2\frac{1}{3}$ atm. for the first time from 10 p.m.—2 a.m. without any trouble. Half-an-hour after his ascent he was seized with intense pains in the limbs, with great difficulty in breathing. He soon could not stand and lost consciousness. There was now great dyspnoea, an intermittent pulse, cyanosis of the face, and fine rales over the lower lobes of the lungs. The face became livid, the pulse almost imperceptible. After ether injections and artificial respiration consciousness returned. The patient complained of great pain, and especially in the right arm, and of feeling cold. He could not move. Temperature 36° C. Profuse sweating. The respiration was costal, and the diaphragm fixed in the expiratory position. The man gradually recovered, but 2 months later there was still some loss of motor power, patches of hyperalgesia, increased knee jerks, pains in right elbow and left knee, loss of sexual power, and inability to hold urine more than 1 hour. There was evidently a lesion above the origin of the phrenics in this case, producing the immobility of the diaphragm and arm symptoms. Probably there was another lesion in the lower dorsal region.

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[1] The Blackwall and other Subaqueous Tunnels. *Journ. Soc. Arts*, May 15, 1896.
[2] *Prager med. Wochenschrift*, XXIV. 1899, Nr. 14.

The following are some of v. Schrötter's cases.

Initial symptoms	More or less lasting symptoms
1. Pains in the ears ; intense pains in legs.	Myalgia.
2. Intense pains in all limbs.	Arthralgia and myalgia.
3. Paraplegia of legs; pains in left arm.	Monoplegia of left arm.
4. Pain in chest; vertigo ; difficulty of breathing ; weakness in the legs ; loss of consciousness.	Paraplegia of legs.
5. Vertigo ; pains in limbs ; spastic paralysis of legs.	Paraplegia of legs.
6. Vertigo ; vomiting.	Ménièr's symptoms.
7. Loss of consciousness and convulsions; vertigo; deafness.	Ménièr's symptoms ; aphasia.
8. Vertigo ; deafness ; double vision ; maniacal fits.	Apoplectiform ; deafness ; Ménièr's symptoms.
9. Paraplegia ; asphyxial phenomena ; loss of consciousness.	Spastic paraplegia.

Schrötter noticed brachycardia in several cases, disappearing within at most three weeks of the accident. The pulse rate diminished by 18 to 42 beats per minute. He attributes the cause of this to air emboli causing central stimulation of the vagus, or to air bubbles in the coronary circulation interfering with the nutrition of the heart. Either cause has been proved experimentally to decrease the frequency of the heart.

In the case of diver's sickness a large number of reports have been gathered together by Bert. M. Denayreuze[1] reported on 200 men who dived to a pressure of $+3$ to $+4$ atm. During six months' work five men died and a great number were affected, most commonly with paralysis of the legs and bladder and deafness. The men who quickly returned to the surface suffered most. None of the deaths occurred while the men were under water. M. Gal recorded the following typical cases occurring among the Greek divers.

I. Submerged for 15′ at 40—45 metres ($+4$ to $4\frac{1}{2}$ atm.). Some minutes after returning to the boat the diver complained of dizziness and fell down and died.

II. Submerged for 45′ at 40 metres ; 15′ after being pulled up he was seized with pains, and almost at once lost consciousness and died.

III. 30′ after return to the boat was seized with severe pain in the epigastrium. Became paralysed in legs, bladder, and rectum. Died after 3 months.

IV. Became paraplegic shortly after return to boat. Cured in 3 months.

[1] *loc. cit.* p. 413.

V.　Depth 35—45 metres; paralysis of legs: cured in 5 days.

Messrs Siebe and Gorman tell us that among the Greek divers about a score of lives are lost every year. Catsaras[1] has recorded many such cases, and among others cases of motor aphasia, sensory aphasia, hemiplegia, Jacksonian epilepsy, blindness, and vertigo.

Through the agency of Messrs Siebe and Gorman we circulated among the pearl divers of Australia questions concerning the illnesses of divers. The divers there go to 100 feet and very rarely to 125 feet. In shallow water they work for two hours, in deep water for 15′—20′.

The symptoms of illness occur directly or soon after the diver comes on deck. "There is no pain whilst on the bottom but directly the diver comes to the top." The pains occur in the hips and knees, calves of legs and arms. Paralysis of the leg and incontinence of urine are the graver symptoms. Bassett Smith of H.M.S. Penguin[2] says cases of slight paralysis are very common. The men are paid by results, and so will go to considerable depths. A local swelling of the abdomen is regarded as of evil omen, and death is supposed to follow if the swelling passes under the ribs. So the treatment is to sit on the swelling.

The following are extracts from the records of the autopsies which have been made after deaths due to caisson illness and diver's paralysis.

Autopsies of Caissoniers.

(1)　General congestion of the viscera, patches of congestion on the brain.

(2)　General subcutaneous emphysema. Congestion of viscera and especially of lungs. A heavy man aged 40. (Pol and Watelle.)

(3)　Interlobar emphysema of lungs, numerous punctated ecchymoses on the pleura and pericardium, bubbles of gas within the blood vessels. Death from bursting of caisson. (Gallard.)

(4)　Softening of some inches of the spinal cord in the dorsal region. (Bert.)

(5)　Extravasation of blood upon the spinal cord opposite the two lower dorsal vertebrae. (A. Smith.)

(6)　Extravasation of blood upon, and softening of spinal cord, haemorrhages in the kidneys. (Jaminet.)

(7)　Death 15 days after decompression. Numerous foci of haemor-

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[1] *Arch. de Neurologie*, 1890, p. 48.
[2] *Lancet*, 1897, i. 309.

rhages and signs of acute myelitis. Small irregular fissures in mid-dorsal cord, filled with round cells. From their well-defined edge probably produced by escape of gas. (v. Leyden.)

(8) Death 2½ months after decompression. Disseminated myelitis in dorsal region with fissures suggesting laceration of the tissues. (Schultze.)

(9) Congestion and oedema of the lungs (Schrötter).

Autopsies of Divers.

(10) Necrobiosis and haemorrhages in or upon spinal cord. (Gal, Catsaras, Greek divers.)

(11) Haemorrhages in or upon spinal cord. (Blick, Australian pearlers.)

Theories of compressed air illness.

The phenomena observed in local compression of the body and in cupping, the pallor of caissoniers, the nose-bleeding sometimes seen on decompression, the congestion of the viscera recorded in a few autopsies, have led most medical men who have observed caisson sickness to suppose that the blood is driven from the exterior and compressed within the viscera.

"Pulmonary and cerebral congestions are," say Pol and Watelle, "the chief results of compressed air. Just as a lowered atmospheric pressure brings blood to the exterior and causes haemorrhages, so does compressed air congest the viscera." The brain and spinal cord, according to Babington, shut up in their osseous cavities, are not able like other elastic parts, to quickly accommodate themselves to changes of pressure.

Bouchard put forward the extraordinary theory that as the gases of the intestine are compressed the abdominal wall becomes concave, and as the abdominal wall resists this distortion it converts the abdomen into a kind of cupping-glass. This leads to congestion of the abdomen during compression, and the reverse on decompression.

A. H. Smith may be quoted as the chief exponent of the mechanical congestion doctrine. He deduced "the law that under high atmospheric pressure the centres will be congested at the expense of the periphery ...and that firm and compact structures will be congested at the expense of those more compressible. Moxon[1] in his Croonian Lectures said "it

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[1] Lancet, 1881, I. p. 528.

needs no experiment to show that great increase of atmospheric pressure must drive the blood away from the surface of the body."

The neglect by these writers of physical laws is the less excusable seeing that Poisseuille[1] in 1835 observed the capillary circulation in frogs, and young mice, enclosed in a glass chamber and submitted rapidly to + 2 to + 8 atm. The circulation continued unaffected. Moreover Paul Bert in 1881 clearly stated the physical error contained in such theories. The body of a workman exposed to compressed air supports according to Guérard

> at + 1 atm. an additional 15,500— 20,600 kilograms
> at + 3 atm. „   „   46,500— 60,800   „
> at + 6 atm. „   „   93,000—123,600   „

If it were not for the incompressibility of the fluids of the body, and the equal and instant distribution of the pressure to all parts, life would be impossible under *any* variation of atmospheric pressure. The fact that mere mechanical pressure is of no importance is shown not only by Guérard's figures but by the existence of abundant life in the sea at depths of 2000 metres, corresponding to a pressure of + 200 atm. The only mechanical compressions which can take place are that of the membrana tympani, which is relieved by opening the Eustachian tube, and that of the intestinal gas. The latter leads to the workmen tightening their belts and to a freer descent of the diaphragm in respiration. It has astonished us to find the most experienced practical men in diving and submarine engineering unaware of the experimental work of Paul Bert, of the conclusion which he drew as to the causation of compressed air illness, and of the precautions which he laid down for the guidance of caisson workers.

Paul Bert by his remarkable experiments, published in 1878, proved that the true cause of caisson sickness is the effervescence of gas in the blood and tissue juices. This explanation had, it is true, been advanced by several authors, such as Hoppe (1857), François, Rameau, Bucquoy, etc., but generally as a cause additional and subsidiary to mechanical congestion.

Bert found by analysis of the gases of the blood that the nitrogen increases in compressed air more or less according to Dalton's law. He found that this gas was set free on rapid decompression, and produced embolism, in the lungs, the central nervous system, etc.: and that the gravity of the result depended on the height of the pressure, the length

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[1] *C. R. Acad. des Sci.* II. 554.

of exposure, and the rapidity of decompression. He also proved that the gas set free in the tissues might produce local swellings and emphysema.

The truth of Bert's explanation is borne out by the varied nature of the lesions found in caisson sickness, by the subcutaneous emphysema which has been detected in a few cases, and by the autopsies which have been recorded. One man is struck with pain in the joints and muscles, and another with respiratory embarrassment and loss of consciousness, another with deafness and vertigo, and yet another with paraplegia. All becomes clear if the cause is once accepted to be local embolism or compression by air bubbles. Thus air bubbles in the posterior roots or posterior columns of the spinal cord may cause the intense pains so often experienced. Air frothing in the heart may kill one man, air in the heart or in the vessels of the lung, or in the respiratory nervous system, produce in another difficult breathing; air embolism in the brain may cause aphasia, hemiplegia, loss of consciousness or sudden death, or in the dorsal cord paraplegia, while a bubble in a semicircular canal will explain the cases of auditory vertigo, and bubbles in the joints and muscles cause local swellings and emphysema coupled with pain.

Bert also found that high oxygen tension acts as a general protoplasmic poison arresting metabolism, depressing the body temperature, and causing the discharge of convulsions in mammals, and finally the death of all forms of life.

Since the publication of Bert's results little experimental work has been done on the subject. The most important paper is one by Lorrain Smith[1], who has found that high oxygen tension produces inflammation of the lungs. During the last few years we have been reinvestigating the effects of compressed air and oxygen, and we propose to communicate the results obtained so far, under the following headings.

1. Effects on the respiratory exchange and body temperature.
2. Effects on the nitrogen output.
3. Effects on the lungs.
4. Effects on the neuro-muscular system.
5. Effects on the central nervous system.
6. Effects on the blood gases.
7. Effects on the circulation.
8. The effects of decompression.
9. The rules of safe working for caissoniers and divers.

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[1] *Journal of Physiology*, xxiv. p. 14. 1899.

Effects on the Respiratory Exchange and Body Temperature.

Bert enclosed animals in a small air-tight chamber and exposed them to increased pressures of air or oxygen. The chamber was not ventilated, and the animals were left till they died, when the air in the chamber was analysed.

In ordinary air the animals died from want of oxygen when the tension of oxygen fell to $3 \cdot 4 \%$ atm.

In super-oxygenated air or in air compressed to $+0$ to 4 atm. they died from CO_2 poisoning when the tension of CO_2 rose to 25% of an atm. But when the tension of O_2 was raised above 150% atm. (over 7 atm. air) the animals died before the CO_2 tension reached 25% of an atm. This was due to the *toxic effect* of oxygen. The higher the oxygen tension the more rapidly died the animals, and the less CO_2 was produced.

Bert also observed in the rat the following consumption of O_2 and output of CO_2 under varying tensions of oxygen.

O_2 tension	O_2 consumed in 24 hrs.	CO_2 output in 24 hrs.
21 $\%$ atm.	12·6 litres	7·06 litres
48·7 ,,	13·72 ,,	10·32 ,,
87·5 ,,	11·35 ,,	6·96 ,,

Thus an increase of oxygen tension corresponding to $2 \cdot 3$ atm. air slightly increased the respiratory change, while an increase corresponding to $4 \cdot 2$ atm. air slightly diminished it. In frogs Bert obtained the same results.

We have studied the respiratory exchange in animals placed in a pressure-chamber and ventilated with a current of air or oxygen. The chamber was fitted with thick glass windows and a pressure-gauge. It was connected by an inlet tube with a bottle of compressed air or oxygen (Brin's), while an outlet tube, controlled by a screw tap, was connected with a set of Haldane-Pembrey absorption tubes and a gas meter. The outputs of CO_2 and H_2O were obtained by weighing the absorption tubes. The animals were given a wool bed to prevent their losing heat directly to the metal walls of the chamber, and the chamber in some cases was heated on a sand-bath, and the temperature of the outgoing air taken.

In the *Journal of Physiology* (XXIX. p. 492, 1903) we have published examples of the results we have obtained from mice. We concluded that compressed air at a pressure of $+4$ atm. and upwards diminishes the CO_2 output and lowers the body temperature of mice. This effect

generally increases as the pressure rises, but the individual power of resistance differs greatly in different animals. Paul Bert says that "the absorption of oxygen and elimination of carbonic acid diminish in proportion as the tension of oxygen rises, and that an animal breathing pure oxygen at 2, 3, 4 atm. is in the same condition as another breathing air at pressures of 10, 15 and 20 atm.[1]"

We observed like Bert that prolonged exposure to even one atmosphere of pure oxygen slightly lowered the CO_2 output and temperature of mice, both returning to the normal on replacing the oxygen with air[2]. On increasing the tension of oxygen we find a general increase in toxic effect but no constant relationship owing to differences in the individual susceptibility of mice. For with mice kept at the same tension of O_2 and the same external temperature one may be affected sooner than another.

According to Bert's conclusion we expected to find that increased *air* pressure would depress the CO_2 and H_2O output in proportion to the partial pressure of oxygen. But this has not been the case. We have found 10 atm. of air to be as a rule far more depressing than 2 atm. of oxygen, and there are other factors to be considered besides the partial pressure of oxygen. These are the cooling effects of compressed air, and the resistance which highly compressed air may establish (1) to the movement of the air in and out of the air tubes, (2) to the diffusion of CO_2 from the alveolar air into the tidal air. We have no evidence to offer as to the relative importance of these two factors.

The increased loss of body heat arises firstly because the compressed air is a better conductor of heat, and secondly because the compressed air becomes saturated with water.

If the chamber be ventilated at the rate of 1 litre of air at atmospheric pressure per minute, and the compressed air be saturated with water vapour, the amount of water carried away per litre will fall to $\frac{1}{2}$, $\frac{1}{3}$, $\frac{1}{4}$ etc. as the pressure rises to 2, 3, 4 atms. The chamber and the animal thus become wet with condensed water vapour, and it is not possible to measure the water output of the animal by analysis of the ventilation air.

Rubner observed that an arm enclosed in a calorimeter and clothed in dry flannel lost 4·5 cal. per hour, while when the flannel was wet it lost 22·7 cal., an increase of 344 %. Thus when the chamber is cold the dampness of the animal's fur may greatly increase the heat-loss,

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[1] *La Pression Barométrique*, p. 612.

[2] For details cf. *Proc. Roy. Soc.*, 1902, Vol. LXX, p. 455.

for, owing to the skin temperature being much greater than that of the air, the evaporation of water from the body of the animal is not hindered by the saturation of the air with water vapour. At very high temperatures 85—90° F. the saturation of the air with moisture would so lessen the evaporation of water from the body as to render a caisson-worker in danger of heat-stroke.

Dr Haldane tells us that miners, almost stripped, can work in air saturated with water at 85° F. and, for short shifts, even at 93° F. without rise of body temperature. They pour with sweat and drink copiously. A man clothed in flannel, on the other hand, will rise to 103° F. or higher on walking in the mine under these conditions. Miners as a rule begin to be cautious of air above 85° F. and saturated with vapour. The explanation of these facts is given in the following figures. At 20° F. air takes up 1·3 grains of water per cubic foot, at 60° F. 5·77, at 85° F. 12·78, at 93° F. 16·21, and at 99° F. 19·28.

Experimenting with cold wet air we have found that mice may have their metabolism increased by a current of moist air at 20° C., and that finally by prolonged exposure their body temperature may be lowered and death result from the increased heat loss.

We have repeated the observations made on mice, on rats and young rabbits with the following results.

I. *Large rat.*

Time	Atm. O_2	CO_2 output per min.	Remarks
11.15 a.m.	$+3—3\frac{1}{2}$		Room temp. 15·5° C.
12.30 p.m.		·00618	Body ,, 37° C.
			Decompressed at 12.55, rat all right, body temp. 37° C.
1 ,,	$+4$		
2 ,,	,,	·0870	
2.45 ,,	,,	·00663	
3.20 ,,	,,	·00653	

At 3.53 the rat was decompressed in about 1 minute. Spasms and cyanosis followed owing to gas embolism. The body temp. was 35° C. The blood contained many bubbles of gas.

II. *Large rat.*

Time	Atm. O_2	CO_2 output per min.	Remarks
10.22 a.m.	$+3—4$		Room temp. 19·5° C.
1.55 p.m.	,,	·00894	Body ,, 37° C.
3.15 ,,	,,	·00582	
3.40 ,,	,,	·00558	
4.30 ,,	,,	·00376	

Decompressed in 10 mins. at 4.50 p.m. Rat very inert, slight spasms, body temp. 30·5° C. Some gas bubbles in the blood.

III. *Young rabbit.*

Time	Atm. O$_2$	CO$_2$ output per min.	Remarks
10.54 a.m.	1	·0074	
11.25 ,,	+2·5—3		
11.45 ,,	,,	·0057	
12.50 p.m.	,,	·00693	
1.20 ,,	,,	·00637	
2 ,,	,,	·0069	
2.40 ,,	,,	·00475	

After slow decompression the rabbit seemed all right. The body temp. was 36° C.

IV. *Large rat, weight 225 grms.*

Time	Atm. O$_2$	CO$_2$ output per min.	Remarks
10.55 a.m.	+5		Room temp. 22° C.
12 p.m.	,,	·0062	
12.20 ,,	,,	·0061	
1 ,,	,,	·0	

On decompression the rat was found moribund. The body temp. was 28° C. The lungs very congested.

V. *Large rat.*

Time	Atm. O$_2$	CO$_2$ output per min.	Remarks
11 a.m.	+4		Body temp. 37° C.
11.10 ,,	,,	·0072	Room ,, 19° C.
12.10 p.m.	,,	·003	
12.20 ,,	,,	·0013	

On decompression animal found moribund. Body temp. 29° C.

VI. *Large rat, weight 165 grms.*

Time	Atm. O$_2$	CO$_2$ output per min.	Remarks
10.50 a.m.	+2—3		Room temp. 18° C.
11.55 ,,	,,	·0087	Body ,, 37° C.
12.10 p.m.	,,	·0068	
12.45 ,,	,,	·0107	
2.5 ,,	,,	·0079	
2.45 ,,	,,	·0054	

Decompressed slowly ; animal seemed all right. Body temp. 36° C.

VII. *Young rat.*

Time	Atm. O$_2$	CO$_2$ output per min.	Remarks
10.35 a.m.	+3—4		Room temp. 20° C.
10.45 ,,	,,	·0058	Body ,, 37° C.
12.10 p.m.	,,	·0035	
12.30 ,,	,,	·0042	
1.35 ,,	,,	·0033	
2 ,,	,,	·0036	
2.55 ,,	,,	·0013	

Decompressed. Rat moribund. Body temp. 29° C.

VIII. *Large rat, weight* 152 *grms.*

Time	Atm. O_2	CO_2 output per min.	Remarks
11.10 a.m.	$+3\frac{1}{2}$		Body temp. 37·4° C.
11.30 ,,	,,	·0056	Room ,, 18·5° C.
11.45 ,,	,,	·0055	
12.47 p.m.	,,	·0049	
1.13 ,,	,,	·0049	
1.53 ,,	,,	·0038	

On decompression the rat was inert and the rectal temp. 32·5.   Blood collected in conical glass and quickly analysed. CO_2 67 %.   Temp. of water bath 18·5° C.

IX. *Large rat.*

Time	Atm. O_2	CO_2 output per min.	Remarks
11.25 a.m.	$+3$		
1.15 p.m.	,,	·0077	
2.17 ,,	,,	·0062	
2.24 ,,	,,	·006	
3.20 ,,	,,	·0054	

Slow decompression.   Rat all right.   Body temp. 36·5° C. CO_2 in blood 59·3 %.

X. *Large rat, weight* 139 *grms.*

Time	Atm. O_2	CO_2 output per min.	Remarks
10.30 a.m.	$+4$	—	Room temp. 16·5° C.

Decompressed at 12.30.   Rat all right.   Body temp. 35° C. CO_2 in blood (collected under petrol) 45·8 %.

XI. *Rat, weight* 115 *grms.*

Time	Atm. O_2	CO_2 output per min.	Remarks
1.20 p.m.	$+5$		Body temp. 36·6° C.
1.58 ,,	,,	·0045	
2.39 ,,	,,	·005	
3.10 ,,	,,	·0052	
3.35 ,,	,,	·0047	
3·56 ,,	,,	·0038	

The rat on decompression seemed somewhat inert and dull.   Body temp. 30° C.

XII. *Rat, weight* 121 *grms.*

Time	Atm. O_2	CO_2 output per min.	Remarks
10.30 a.m. 12.30 p.m.	$+4$ atm. falling to $+1$ atm.		
12.30 ,,	$+6$ atm.		
1.54 ,,	,,	·0076	
2.27 ,,	,,	·0049	

Slow decompression.   Rat very inert.   Temp. 28° C.

XIII. *Rat.*

Time	Atm. O$_2$	CO$_2$ output per min.	Remarks
10.30 a.m.	+6		
11.15 ,,	,,	·0061	
12.15 p.m.	,,	·0078	
1.7 ,,	,,	·0041	

Body temp. on decompression 30° C.   Rat inert.

XIV. *Rat, weight 115 grms.*

Time	Atm. O$_2$	CO$_2$ output per min.	Remarks
10.45 a.m.	+5	—	
12.59 p.m.	,,	·0024	

Body temp. on decompression 34° C.   Rat quiet, otherwise well.   CO$_2$ in blood (petrol method of collection) 71·8 % and 71·2 %.

XV. *Young rabbit.*

Time	Atm. O$_2$	CO$_2$ output per min.	Remarks
12.15 p.m.	+6		
12.35 ,,	,,	·0088	
1.50 ,,	,,	·0023	

Moribund on decompression.   CO$_2$ in blood (oil method of collection) 66·41 % and 65·31 %.

The above series of experiments show that :—

Some rats or rabbits can tolerate	Some rats or rabbits are rendered moribund by
Atm. O$_2$ +2—3 for 3—4 hrs.	Atm. O$_2$ +3—4 in 4½ hrs.
,, + 3 ,, 4 ,,	,, + 4 ,, 2½ ,,
,, + 4 ,, 2 ,,	,, + 5 ,, 2—3½ ,,
	,, + 6 ,, ½—3 ,,

The CO$_2$ output is depressed and the body temp. falls just as in mice.

The resistance to O$_2$ poisoning also differs in individuals very considerably, but the larger animals seem just as susceptible as mice. As the chamber used in these experiments was not provided with a window, we were unable to observe the symptoms of oxygen poisoning.

After exposure of dogs to high tension O$_2$ and *rapid* decompression Bert found the animals convulsed.   Analysing the blood-gases during the convulsions he found the CO$_2$ diminished to 14·8% and 10·5%.   He concluded that the oxygen had arrested the tissue metabolism.   The circulation must have been most imperfect in these animals owing to gas embolism.

In some of the above experiments we analysed the CO_2 % in the blood at the time when the CO_2 output was diminished and the body temperature lowered. Our method was to decapitate the animal after decompression in 10′, allow the blood to drop under oil in a conical glass, take up the blood in a syringe containing oxalate solution, and analyse two samples (each of 1 c.c. vol.) by the Haldane-Barcroft apparatus. We omitted the ferricyanide process as we did not want to measure the oxygen.

It will be seen that our CO_2 results by this method have been very high, and considerably higher than controls done with the gas pump. We have recently discovered that the omission of the ferricyanide process increases the volume of gas displaced by the tartaric acid. The acid turns out some of the oxygen. Further enquiry into the causation of this we defer for the present. From normal rats we obtained CO_2 values such as the following : $- 52.41$ %, 63.27 %.

From an asphyxiated rat we obtained 68.21 %.

From two rats exposed to, but not seriously affected by, compressed O_2 we obtained 45.8 % and 59.3 %, while from two others rendered moribund we obtained 66 % and 71.8 %. The results do not confirm those of Bert but show that the CO_2 content of the blood of an animal poisoned by O_2 resembles that of an asphyxiated animal. They show that the blood does not contain less CO_2, in spite of the diminished output of CO_2 from the lungs. The diminished CO_2 output might be attributed to the inflammation of the lung which is produced by compressed O_2. This inflammation might be supposed to lessen the respiratory exchange. We think, however, that the lowering of the body temperature clearly proves diminished tissue oxidation ; moreover, the lessened CO_2 output occurs before the pneumonia is set up. The slow and feeble outflow of blood in the O_2 poisoned animals shows how feeble the heart, and how imperfect the circulation of the blood have become. The veins are distended, and the blood is mostly in the venous side ; very little flows from the severed carotids. Hence the amount of CO_2 in the blood is great in spite of the diminished production by the tissues.

Effects on the nitrogen output.

Bert compressed himself to a little less than $+1$ atm. for about 3 hrs. a day, and obtained the following results.

		Urine	Urea
1st day	Normal pressure	1650	20·15
2nd ,,	Compressed	2010	24·72
3rd ,,	Compressed	1990	26·04
4th ,,	Compressed	2255	21·18
5th ,,	Normal pressure	2080	20·80
6th ,,	,, ,,	2150	22·50

A dog placed on a daily ration and catheterised gave

		Urea
1st day	Normal pressure	7·9
2nd ,,	3 atm.	10·4
3rd ,,	,,	9·0
4th ,,	Normal pressure	9·1
5th ,,	,, ,,	8·4

From these experiments Bert concludes that moderate pressures increase the nitrogenous metabolism. Snell by observation on himself at the Blackwall tunnel was unable to determine any effect on the urea output. In two dogs compressed to 8 atm., Bert determined a considerable decrease in the urea output.

The details of Bert's experiments are as follows.

Dog. 12 kilo. Fed daily at 7 a.m. on 250 grm. bread and 250 grm. meat and 500 grm. water.

July 25. Catheterised at 8 a.m. Experiment begins.

July 26. Catheterised at 8 a.m. 280 c.c. urine; 12·1 grm. urea. Compressed to 8 atm. from 9 a.m.—3 p.m. Decompressed 3—5 p.m. Rectal temp. 35·5. Animal appears well.

July 27. Only half its ration eaten. 350 c.c. urine; 3·7 grm. urea!

July 28. No food eaten. 510 c.c. urine; 10·3 grm. urea.

Dog. 16 kilo. Same diet.

Aug. 3. 8.30 a.m. catheterised.

Aug. 4. 8.30 catheterised. 475 c.c. urine; 21·6 grm. urea. Temp. 35·8. Put at 8 atm. from 9 a.m.—4.50 p.m. Decompressed 4.50 − 6.20 p.m. Temp. 35·5. Animal seems well.

Aug. 5. 245 c.c. urine; 16·9 grm. urea.

It will be noticed that Bert only determined the urea for one day previous to compression. This is not a sufficient control. The fall from 12·1 to 3·7 grm. in the first experiment must surely be due to some error.

We have observed the urine of three dogs exposed to eight atmospheres of air. The dogs (females) were prepared for catheterisation by slitting the perineum. They were then placed in a metabolism cage and put on a weighed and constant diet of dog biscuit and milk—more

than sufficient to supply their energy. The bladder was emptied each morning with a catheter and syringe, and the urine analysed. After several days the animals were placed in the pressure-chamber and exposed to 8 atmospheres for 6—7 hours. Decompression was carried out in 2 hours so as to avoid all risk of air-embolism, and the animals were then returned to the metabolism cage for the night. A cage was fitted up within the pressure-chamber so that the urine, passed during compression, could be collected.

Experiment 1. The dog was kept for four days at normal atmospheric pressure. On the fifth day the pressure was raised to 100 lbs. to the sq. inch (almost +7 atm.) and kept at this pressure for 5 hours. Three hours were then taken to decompress the animal. In the urine of this day there was no definite change. During the next two days the dog was kept at normal pressure and the urine showed a slightly increased amount of nitrogen. On the eighth day the dog was exposed for seven hours to 100 lbs. pressure. The weather was very cold and we tried to warm the chamber by applying a Bunsen burner to the outside. During the fourth hour the dog became restless and tore away some of the wire netting at the door of the cage. It also defaecated in the cage and fouled its fur so that some of the urine may have been lost.

On the next day the animal was again put under 100 lbs. pressure. In about the third hour it became very restless and tore down the wire netting of the cage and escaped into the chamber. Shortly after this the dog began to profusely salivate, while the respirations became jerky in character. The pupils dilated and the eyelids twitched. At the end of six hours decompression was begun. It was completed in 2¼ hours. The dog was cold on removal, wet with saliva, and with moisture taken up from the water-saturated air of the chamber. Its respirations were jerky and somewhat embarrassed with a frequency of 62. The animal would not eat.

During the next few days the dog was kept in the metabolism cage and recovered completely.

The nitrogen content of the urine was distinctly increased on the ninth day when the above described symptoms occurred. The increase persisted on the following day. The rise in nitrogen output is quite remarkable considering that the animal took no food on the 10th day, and only milk on the 11th day. The experiment is contrary to those of Bert.

We consider that the increased N output was due to the restless efforts of the dog to open the cage, i.e. to excessive muscular work.

Experiment 2. The dog died on the second day under pressure and so rendered this experiment incomplete.

After five days' preliminary observation of the nitrogen output, the dog was submitted to 6—7 atm. of air from 11 a.m. to 7 p.m. The weather was cold (February). At 4 p.m. the dog commenced to salivate—there were no other symptoms. On the next day it was again placed under the same pressure. It salivated profusely and was very restless. Two hours before its removal from the cage the dog lay quiet. On removal, after slow decompression, it manifested

EXPERIMENT I.

Day	Food		Quantity of urine	Reaction	Sp. gr.	Total N	Urea N	SO_3	P_2O_5	Sugar	Albumin	Weight of dog	Atm. pressure of air
	Milk	Biscuit											
1*	284 c.c.	one	350 c.c.	acid	—	—	1·480	1·0013	·343	nil	nil	4803	+ 0
2	„	„	325 „	„	1011	1·82	1·437	—	·2925	„	„	—	„
3	„	„	500 „	neutr.	1005	1·372	1·1020	·1080	·270	„	„	4830	„
4	„	„	400 „	faint alk.	1010	1·872	1·324	·1388	·280	„	„	—	+6—7 atm. for 6 hrs.‡
5	„	two	460 „	„	1010	1·584	—	·1500	·212	„	present	4950	+0
6 & 7	568 c.c.	one	550 „	„	1017	2·31†	1·990†	·2453†	·253†	„	„	—	+6—7 atm. for 7½ hrs.;‡
8	284 c.c.	„	505 „	„	1010	1·960	1·960	—	·200	„	„	4760	7 hrs.‡
9	„	„	only catheter specimen obtained	acid	—	2·44 %	2·15 %	—	—	„	„	—	„
10	none	none	168 c.c.	„	1020	2·63	2·217	—	·218	„	nil	4803	+ 0
11	284 c.c.	one	210 „	„	1017	2·247	2·003	—	·170	„	„	4860	„
12	„	„	173 „	„	—	1·951	—	—	·242	„	trace	—	„

* The analysis for each day is that of the urine passed the *previous* day.
† For 24 hrs.   ‡ In addition 2 hrs. were spent in slow decompression.

EXPERIMENT II.

Day	Food		Amount of urine	Sp. grav.	Total N	P_2O_5	Atm.
	Milk	Biscuit					
1	284 c.c.	one & a half	334 c.c.	1010	2·1	0·398	+ 0
2	„	„	370 „	1009	1·92	0·490	„
3	„	„	336 „	1018	2·114	0·393	„
4	„	two	300 „	1015	2·268	—	+6—7 „ for 8 hrs.*
5	„	„	312 „	1015	2·0	0·3062	+6—7 for 6 hrs.
6	„	„	275 „	1012	2·41	0·2674	(died after decompression)
7	„	„	375 „	—	1·942	0·1787	
8	„	„	100 „ ,+	—	0·658 %†		

* Decompression was carried out in 2 hrs.   † In 8 hrs. before death ($\times 3 = 1·974$).

Experiment III. *Metabolism.*

Day	Food Milk	Food Biscuit	Amount of urine	Sp. gr.	Reaction	Total N	Urea N	SO_3	P_2O_5	Atm.
1	284 c.c.	one & a half	330 c.c.	1013	faintly acid	2·821	—	0·336	—	+ 0
2	,,	,,	330 ,,	1013	,,	2·402	1·742	0·208	—	,,
3,4	,,	,,	320 ,,	1014	,,	—	1·869	0·292	0·512	,,
5	,,	,,	265 ,,	1018	faintly alk.	2·114	1·860	0·252	0·450	6 hrs. in +6—7
6	,,	,,	250 ,,	1023	,,	3·272*	2·829	0·212	0·750	+ 0
7	,,	,,	253 ,,	—	,, acid	3·080	2·535	0·253	0·483	,,
8	,,	,,	215 ,,	1018	acid	2·660	2·073	0·239	0·430	
9,10,11	,,	,,	163 ,,	1024	faintly acid	2·647	2·565	0·294	0·465	,,
12	,,	,,	200 ,,	1020	faintly alk.	2·816	2·236	0·226	0·584	6 hrs. in +6—7
13	,,	,,	153 ,,	1022	,,	2·270	1·958	0·185	0·283	+ 0
14	,,	,,	208 ,,	1018	faintly acid	2·780	—	—	—	,,
15	,,	,,	225 ,,	1017	faintly alk.	2·475	2·137	0·266	0·448	,,
16,17,18	not done	—	—	—	—	—	—	—	—	
19	284 c.c.	one & a half	246 ,,	1020	faintly alk.	2·917	2·460	0·299	0·492	,,

* Urine was cloudy and contaminated with food.

symptoms of dyspnoea and died 20 minutes later. Death resulted from gas embolism in spite of slow decompression, owing, we believe, to the oxygen poisoning and pneumonia which had developed in this dog. Only 100 c.c. of urine could be obtained during the eight hours' observation of this day. This contained 0·658 % N.

No change in the nitrogen output is to be detected in this animal.

Experiment 3. The dog was exposed to +6—7 atm. for six hours on the 6th day and again on the 12th and 13th days of observation. On the 13th day the dog seemed restless towards the end of the period of compression.

No noteworthy change in the urine occurred.

The three experiments here recorded show that there is no marked and constant variation in the urinary constituents of dogs submitted to + 6—7 atm. for several hours. In the case of the first dog, there was, it is true, a definite increase in the output of nitrogen. This we are inclined to attribute to the continuous and violent efforts of the dog to escape from its cage. A similar increased output of nitrogen would probably follow the convulsions due to oxygen poisoning. It is evident from Experiment III. that a dog can be exposed to + 6—7 atm. for six hours without alteration of its urinary constituents, and we cannot confirm Bert's two experiments conducted on dogs at the same pressure.

Effects on the lungs.

While studying the oxygen tension of the blood by the CO method of Haldane and Lorrain Smith, the latter[1] found "that exposure of animals to a tension of 170—180 % atm. O_2 causes in a short time diminution in the power of the lungs to *actively* absorb oxygen, and that with a continuance of this exposure the arterial oxygen fell till it reached the level for which mere diffusion of oxygen from the alveolar air might account." He subsequently[2] determined that exposure of mice to high partial pressures of oxygen produces pneumonia. "The tissue of the lungs showed intense congestion in the large and small blood vessels. The alveoli were to a large extent filled with an exudate, which was granular and fibrillated in appearance, but did not give the fibrin stain by Weigert's method, nor with eosin." He found that a pressure of 40 % atm. O_2 for 8 days had no effect: 80 % atm. killed two mice in 4 days with congestion of the lungs, while two other mice survived unharmed.

An average pressure of 125·3 % atm. O_2 killed mice in an average time of 64 hours. 180 % atm. killed in about 24 hours, while 300 % atm. produced inflammation of the lungs in 5 hours.

[1] *Journ. of Physiol.*, vol. xxii. p. 315, 1898.
[2] *Journ. of Physiol.*, vol. xxiv. p. 19.

Our results confirm those of Lorrain Smith.

High partial pressure of oxygen exercises a marked irritant effect on the lungs, producing at first congestion of the alveolar capillaries, and afterwards haemorrhagic exudation and consolidation. To the naked eye the lungs present in the early stages a suffused redness. Patches of more intense exudation occur in the apices and edges of the lungs. At a later stage the congestion passes into typical hepatization, the lungs sink in water and are of a dark purple colour. The pneumonia is patchy if quickly, and universal if slowly developed.

The following tables (p. 425) illustrate the onset of pneumonia at different pressures of oxygen and air.

Lorrain Smith found that 180% atm. O_2 killed in about 24 hours, while 300% O_2 produced inflammation in 5 hours. Our results show somewhat higher powers of resistance. Thus in Experiment 2 the mouse showed no symptoms after 6 hours in 300% atm. O_2, while in Experiment 4 the mouse survived 9 hours' exposure to + 400% atm. O_2.

Lorrain Smith suggests that inflammation of the lungs may be a cause of caisson disease as well as decompression gas embolism. We do not find much in our experiments to confirm this view. The highest pressure hitherto used in caissons is + 3·45 atm. and the men never work for shifts longer than a few hours. It seems to require about 24 hours at + 7 atm. (= 168% atm. O_2) to produce marked symptoms of pulmonary congestion.

We observed no sign of lung trouble in a monkey which was exposed on many days to + 7 atm. for 4—5 hours at a time.

To test whether the pneumonia produced by long exposure to + 7 atm. air was due to the high partial pressure of oxygen, we subjected a group of animals to air containing only 10% of oxygen. The partial pressure of oxygen was thus 84% atm. in place of 168%. The gas was supplied by Brins, and being limited in quantity we were not able to freely ventilate the chamber. Soda-lime was placed within to absorb the CO_2, but in spite of this the CO_2 content of the chamber rose to over 1%, so that we were unable to measure the amount in the sample of air which we drew off into Haldane's small CO_2 apparatus for air analysis. The two cats died after 30 hours' exposure, while two out of three mice which were enclosed in a nest of cotton-wool survived. There was no trace of pneumonia in the lungs of the dead animals and we are inclined to attribute the deaths chiefly to increased loss of heat, and partly to CO_2 poisoning.

Increased oxygen pressures.

	Animal	Atm. of pure O₂	Time under pressure	Symptoms of pneumonia	State of lungs
1.	Mouse	1	6 hours	none	Animal lived
2.	,,	3 (chamber at 30° C.)	,,	,,	,,
3.	,,	3 (chamber at 15° C.)	3 hours	Gasping respirations	,,
4.	,,	4 (chamber at room temp.)	9 hours	,,	,,
5.	,,	5 (chamber at 30° C.)	Died in night, probably after about 12 hrs.	,,	Very congested
6.	Mouse Rabbit Rat Cat	6·2 (room temp.)	Died in 2½ hrs. ,, 1½ ,, ,, 1½ ,, ,, 5½ ,,	,, ,, ,, Gasping respirations and salivation	Intensely pneumonic Pneumonic Patchy pneumonic Congested all over, pneumonic patches at roots of bronchi
7.	Two rats	6 (room temp.)	2 hours	—	Both lungs markedly congested and upper lobe pneumonic

Increased air pressures.

Animal	Atm. air	Time under pressure	Symptoms of pneumonia	State of lungs
Mouse	+ 4 to + 5	Up to 24 hrs.	None	Survived
,,	+ 7	Died in 24 to 30 hrs.	Gasping respiration	Pneumonic
,,	+ 9	6 hours	,,	Survived
,,	+ 10	Died in 1½ hrs.	,,	Congestion
Dog	+ 7	Exposure for 6—7 hrs. on 3 successive days	Salivation, jerky respiration	Survived
,,	+ 7	Two days' exposure for 6—7 hrs.	,,	Pneumonic
,,	+ 7 (chamber warmed)	24 hrs.	Panting respiration	Survived
Cat and kittens	+ 7 Pressure fell to + 5 during night. Chamber not ventilated during night	48 hrs. Two kittens survived but died after decompression	Difficult breathing after 24 hrs.	Intense pneumonia

Effect on the neuro-muscular system.

One of us (L. H.) exposed nerve-muscle preparations—the frog's gastrocnemius and sartorius—in a small chamber to 50—60 atm. O_2. After 1 hour the preparations were decompressed and contraction curves recorded, and compared with curves of control preparations. In the case of the gastrocnemius the curves showed remarkably little difference. The muscle was both directly and indirectly excitable; the rate of conduction in the nerve, the latent period and the form and period of the contraction curve were scarcely altered. The thin sartorius, on the other hand, showed a greatly diminished height of contraction and a prolonged latent period. The frog's heart exposed to the same enormous pressure continued to rhythmically beat for one and even two hours. The size of the contraction only gradually became lessened. After exposure for about an hour and decompression the cardio-inhibitory mechanism was tested. Inhibition by excitation of the sino-auricular junction was readily obtained. Excitation of the vagus, on the contrary, remained without effect. The action of the vagus was proved effective before the period of compression. It is probable then that call-stations are paralysed, while nerve, nerve-endings, skeletal, and cardiac muscle are but slowly affected by high tension oxygen. Paul Bert exposed frogs to $335\,\%$ atm. O_2. The animals appeared to be dead in about 40 hours. The heart continued to beat and the muscles were perfectly contractile. The central nervous system was alone paralysed and no reflexes could be excited.

We also constructed a chamber in which a small muscle lever was placed so that a disc, attached to the end of the lever, rested between the glass windows of the chamber. The disc was placed in the path of the arc light, and its shadow was photographed on a sensitive plate. The plate moved on a traveller at a fast rate. Electrodes were introduced into the chamber by means of an ebonite plug, and the muscle was excited and the curve recorded while the muscle was under pressure. As the curves showed no noteworthy change during the early period of compression, we postponed the further investigation of this matter. One of us (L. H.) has also studied with Dr Waller the effect of compressed oxygen on the action current of nerve. This research is still incomplete.

Exposure to 50—60 atm. O_2 immediately convulsed and then paralysed all the forms of life which were placed in our chamber, such as mice, frogs, worms, insects, centipedes, etc. Oxygen appears

then to be a specific poison for the central nervous system and the lungs.

Effects on the Central Nervous System.

Paul Bert observed that exposure to high tension O_2 produces convulsions in *birds*. The following are some of his results.

Atm.	Tension of O_2	Atm. of air corresponding to O_2 tension	Rect. temp.	Convulsions
1·75	150	7	—	None
3	260	13	—	None
4	300	15	—	Convulsions
20	420	20	—	,,
5	420	20	32	Convulsions, decompressed & survived
5	420	20	37	,,
5	420	20	33	Died in 30 mins.

Lorrain Smith has confirmed these results. Two larks at 300% atm. O_2 had violent convulsions in 13 min. The convulsions continued at short intervals and subsided in about 1 hr.

A tension below 270% atm. O_2 produced no convulsions. The slow and gradual increase of O_2 tension caused congestion of the lungs, and did not excite convulsions. Lorrain Smith found that after prolonged exposure to 140% atm. O_2, no convulsions followed the subsequent exposure to 300% atm. O_2. According to Haldane and Lorrain Smith the normal oxygen tension of the arterial blood of birds is 35—40% atm., while in the air breathed the O_2 tension is 21% atm.

In higher partial pressures of oxygen they found that the oxygen tension of the blood continued to be higher than that of the atmosphere and in about the same ratio. Twelve hours exposure to 140% O_2, on the other hand, lowered the oxygen tension of the arterial blood by 50%. This result Lorrain Smith attributes to the congestion of the lungs set up by the high pressure O_2. The congestion prevents the quick rise of oxygen tension in the blood, and so the convulsions fail to appear.

Bert observed that dogs exposed to high pressure O_2 go into violent convulsions *after rapid decompression*.

For example:

I. Dog. O_2 528% atm. for 45 min. Decompression in 3·5 min. Rectal temp. 30° C. Convulsions and death in 24 hrs.

II. Dog. O_2 385% atm. for 30 min. Decompression in 1·5 min. Rectal temp. 36·5° C. Convulsions—crises lasted about 20 min. Succeeded by muscular tremor. Recovered.

The tetanic convulsions were so intense that the dog could be held up by the foot like a piece of wood.

In only one dog out of many experiments did Bert record ' s'agitant demi-convulsivement' while *under compression*. In all other cases the convulsions only came on after rapid decompression. Rapid decompression from *air* on the other hand did not produce convulsions but embarrassed respiration, paralysis, or death.

Bert analysed the blood gases and found the convulsions became marked when the O_2 tension of the air equalled 400% atm. and the blood contained 30% O_2. As his animals breathed in and out of a sac of oxygen enclosed in the chamber, and the respired air contained a large excess of CO_2 ($8\cdot1\%$ was found in one case ; at 5 atm. this $= 40\cdot5\%$ atm. In another case the tension of CO_2 was $86\cdot2$!), it is impossible to draw conclusions from his analyses of the CO_2 in the blood. The animals must have been rendered comatose with CO_2. He found that blood super-saturated with O_2 up to 30—35 vols. $\%$ had no effect when injected into dogs, and concluded that the symptoms are due not to the amount of oxygen in the blood, but to the saturation of the tissues with free oxygen. Lorrain Smith finds that the blood can be 38% saturated with CO and yet the bird be thrown into convulsions by exposure to 300% atm. O_2. It is therefore not the total quantity of O_2 in the blood but *the tension of O_2 in solution* which is the cause of the intoxication. Lorrain Smith found that convulsions were produced in mice by much higher tensions of O_2 than in birds. A tension of 450% atm. O_2 convulsed 2 mice in about 20 min., another mouse became dyspnoeic and died without convulsions.

A rat at 268% atm. O_2 showed marked dyspnoea in 5 hrs. and died overnight.

Two mice at 357% O_2 died in 5 hrs. with congested lungs.

Two mice at 230% O_2 showed great dyspnoea in $9\frac{3}{4}$ hrs.; they recovered after decompression.

Two mice at 285% O_2 became dyspnoeic in $3\frac{3}{4}$ hrs. and one died after $8\frac{3}{4}$ hrs. The other recovered on decompression. In none of these animals were there convulsions.

Our numerous experiments on rats and mice confirm those of Lorrain Smith. The animals become as a rule dyspnoeic and gradually pass into coma as their CO_2 output and body temperature fall. Convulsions sometimes occur when the intoxication of the nervous system is sufficiently rapid and intense.

With 3—3·5 atm. O_2 we have not observed convulsions; with

+ 4—5 atm. O_2 they occur, and not infrequently we have observed them in mice, rats, rabbits and cats.

With exposure to + 6—25 atm. O_2 mice and birds quickly become intensely dyspnoeic and comatose and are not convulsed.

On the other hand exposure to 50—70 atm. O_2 instantly throws mice into convulsions resembling those of acute asphyxia, and death rapidly follows.

Our experiments on the effect of rapid decompression from high pressures of oxygen confirm the results of Paul Bert.

The animals show a very striking tendency to strychnine-like convulsions. Sometimes there results marked reflex hyperexcitability: in other cases violent tetanic spasms occur which may be reinvoked by handling the animals.

Experiments on oxygen convulsions.

Animal	Atm. O_2	Onset of con-vulsions	Onset of coma	Remarks
Mouse	+ 3—3·5	none	none	Survived 6 hrs. compression.
Mouse	+ 4·2	12 mins.	—	Died in 2 hrs. 45 mins.
Mouse	+ 4—5	32 ,,	53 mins.	Died soon after onset of coma.
3 mice	+ 4—5	none	about 30 mins.	Decompressed rapidly, spasms fol-lowed.
Mouse	+ 5·5	5 mins.	25 mins.	Died in 35 mins.
2 mice	+ 5·5	20 ,,	—	Survived, comatose for 2 hrs.
Mouse	+ 10	none	10 mins.	Died in 45 mins.
Mouse	+ 25	none	a few mins.	Died in about 10 mins.
Linnet	+ 6	none	about 20 mins.	Rapidly decompressed. Haemor-rhages from diploe and beak.
Linnet	+ 9	none	about 20 mins.	Rapidly decompressed. Haemor-rhage from beak and in diploe.
Rat	+ 5·2	32 mins.	soon after con-vulsions	Died in 57 mins.
Rat	+ 5	none	none	Survived 2 hrs. exposure. Hyper-excitable after decompression.
Rabbit	+ 5·2	17 mins.	45 mins.	Died in about 60 mins.
Cat	+ 5·2	3 hrs. 30 m. one fit only	soon after fit	Salivation began in 3 hrs. 12 mins. Died in 5 hrs. 20 mins.

With air-pressures up to + 12 atm. we have not observed convulsions during compression, the process of intoxication is too gradual.

After rapid decompression animals are often thrown into convulsions owing to the frothing of gas in the heart and consequent asphyxia, or to bubbles of gas set free in the nervous system. The convulsions soon terminate in paralysis. After rapid decompression from oxygen, on the other hand, convulsions continue to be excited, for the oxygen gas set free maintains the life of the tissues.

In the case of compressed air, the chief gas set free is nitrogen, and as this produces anoxaemia the convulsions quickly terminate in paralysis. The convulsions which Bert details as occurring in dogs are clearly decompression results, and due to the effervescence of oxygen gas in the central nervous system. The convulsions which occur during compression are due to the high tension of the oxygen in solution in the tissue lymph. They occur by no means constantly, but only in certain individuals and under certain conditions. Often dyspnoea, coma, and paralysis come on without any marked stage of exaltation. There is one sign of excitement which is almost always present in mice, and that is rapid cleaning movements of the face. The convulsions seem never to occur when the O_2 tension is below 300 % atm. or above 600 % atm., excepting the instantaneous convulsions which precede the death of animals exposed to enormous pressures such as + 60—70 atm. We may assume that with tensions below 300 % atm. O_2, the amount of gas in solution is not sufficient to excite; that with tensions above 600 % atm. O_2 the nervous system is rapidly paralysed by the large dose.

Effect on the blood gases.

Paul Bert analysed the blood gases of a few dogs exposed to compressed air by drawing off samples from the carotid artery as the pressure rose to 2—3—6 etc. atm. He found that the amount of nitrogen became increased, and, if we accept the recent determination of the coefficient of absorption of N_2, the increase is about as required by Dalton's law. The following table shows types of his results. We have

Examples of Paul Bert's blood-gas analyses.

Atm.	O_2	CO_2	N_2	Atm.	O_2	CO_2	N_2	N_2 calculated from pressure, taking coefficient of absorption of N_2 at 37° as 1·23 (Winkler)
1	18·2	37·1	2·2	2	19·1	37·7	3·0	2·46
				5	20·6	40·5	6·4	6·15
1	18·4	47·7	2·5	3	20·0	42·2	4·4	3·69
				6·75	21·0	41·3	7·1	8·3
1	19·4	35·3	2·2	3	20·9	35·1	4·7	3·69
				6	23·7	35·6	8·1	7·38
1	22·8	50·1	2·3	5	23·9	35·2	6·0	6·15
				8	25·4	37·6	9·5	9·84

carried out similar analyses. The animals (anaesthetised) were placed in our large chamber and the carotid artery connected to one of the exit

tubes. On opening the tap a sample of blood could be collected in one of the weighed blood-bulbs of the Hill gas-pump. The air-pressure expelled the blood so forcibly that no precautions to prevent clotting were required. A normal sample was obtained and then other samples at varying times of exposure to compressed air or oxygen. The samples were evacuated by the Hill pump, analysed by the potash pyro method, and the results reduced to 0° C. and 760 mm. The percentage of haemoglobin was also determined in the samples by the Gowers-Haldane instrument. Our results are published in full in the *Journal of Physiology*, Vol. XXIX. 1903, p. 382. The following table gives some typical results.

Examples of blood gas analyses.

Atm.	O₂	CO₂	N₂	Atm.	Duration of exposure	O₂	CO₂	N₂	N₂ calculated from pressure, taking 1·23 as the coefficient of absorption of N₂
1	22·5	32·79	2·03)	+6⅔	30 m.	22·10	37·28	8·41	9·42
			)	+6⅔	2 h. 45 m.	25·81	40·66	11·61	9·42
1	14·2	43·40	2)	+6⅔	1 hr.	14·3	45·7	10·14	9·42
			)	+6⅔	1 h. 30 m.	10·56	39·41	12·9	9·42
			}	+5⅓	45 m.	14·74	37·7	7·48	7·78
				+5⅓	1 hr.	16·55	39·24	9·27	7·78
1	13·7	47	1·53)	+6⅔	1 h. 5 m.	17·4	40·9	11·17	9·42
									O₂ calculated from Hb % and from pressure
				+6⅓ O₂	40 m.	24·2	32	2·16	28·96
			)	+6 O₂	1 h. 35 m.	30·3	37·12	3·13	28·55

In our paper in the *Journal of Physiology* we calculated the nitrogen not according to its coefficient of absorption in water but according to the result of normal blood-gas analyses, and multiplied the atmospheres by 1·8 in place of 1·23. This made the nitrogen found less than that calculated, and we concluded that time was required to fully saturate the arterial blood according to Dalton's law. Regnard and Portier[1] make the same error in regard to Bert's analyses and conclude that his results do not come up to the requirements of Dalton's law. There is always some leakage in blood-gas analyses, and the N₂ given as 1·8 (Pembrey) or 1·5 % (Tissot) is too high. Taking 1·23 as the coefficient of absorption at 37° the figures of Bert and ourselves agree fairly well with Dalton's law. The tissues must obviously take far longer than the blood to become saturated. This explains why short shifts are far less

[1] *Traité de Physique Biologique*, vol. I. 1901.

dangerous for divers and caissoniers. The shorter the shift the less becomes the saturation and the less gas is set free on decompression.

Effect on the Circulation.

Paul Bert recorded the blood pressure in a dog submitted to less than + 1 atm. The blood pressure became higher, the respiratory oscillations augmented, and the respiration less frequent.

We have recorded the blood pressure in dogs, cats, rabbits, while under compressed air and oxygen. One of us (L. H.) at first experimented in the following way. He placed an anaesthetised and morphinised dog at the bottom of a large autoclave, connected the carotid artery with a manometer, and placed the manometer to write on a small drum *enclosed in* the autoclave. Having set the blood-pressure record going, the lid was screwed on and the pressure sent up to two atmospheres by means of an oxygen bottle. Decompression was then brought about, the autoclave opened and the tracing examined. Tracings taken in this way showed that no noteworthy effect on the circulation resulted either from rapid compression or decompression.

We recently repeated this experiment on a chloralised rabbit placed in our large observation chamber. The pressure was raised to 75 lbs., and the effect of oxygen and air were tried in turn. We could detect no change in the mean blood pressure or pulse rate during compression. On sudden decompression there occurred a temporary rise in pressure—probably a reflex effect due to the noise of the escaping air.

We have also observed, with the microscope, the capillary circulation in the frog's web and bat's wing, the animals being placed in a chamber fitted at either end with glass windows. The web or wing was spread over one window and illuminated with the arc light. We could detect no change either in the calibre of the blood vessels, or the rate of flow, when the pressure was quickly raised to + 20—30 atm. Neither did any change occur on rapid decompression, that is, until gas bubbles frothed off from the blood.

These experiments prove then once and for all that the pressure has no mechanical effect on the circulation, and they overthrow all the mechanical congestion theories of caisson-illness.

Effects of decompression.

Out of 24 dogs exposed by Bert to 7—9½ atm. and then rapidly decompressed in 1—4 minutes 21 died from the setting free of gas in the blood and tissues and only 1 escaped without symptoms.

Out of 3 cats exposed to 8—10 atm. and decompressed in 2—3 minutes, 1 died in 15 minutes, and the other 2 became paralysed with softening of the spinal cord.

Three rats exposed to $5\frac{1}{2}$—$6\frac{1}{2}$ atm. survived rapid decompression, while 2 at 8 atm. died.

Bert's experiments on slow decompression.

Animal	Atm.	Duration of full compression	Duration of decompression	Result
Rabbit	10 in 1 h. 30′	5 hrs.	2 hrs.	Temp. fell from 39·6 to 36·7, wet and trembling, survived.
Cat				Temp. fell from 39·5 to 34·3, wet and trembling, survived.
Rabbit	10 in 1 h. 5′	30′	20′	$1\frac{1}{4}$ hr. after decompression became paraplegic, died.
Cat				Convulsions & died, gas in right heart, 15·9 °/₀ CO_2, 84·1 N_2.
Cat				Convulsions and died.
Guinea pig	10 in 1 h. 5′	15′	10—5 atm. in 1′	Paralysis, died, gas in venous system.
Cat			5—1 atm. in 25′	Survived, no symptoms.
Large dog (19 kilos)	$7\frac{1}{2}$ in 1 hr.	$4\frac{3}{4}$ hrs. in $7\frac{1}{2}$—$4\frac{1}{2}$	1 hr.	Wet and cold, dying on extraction, gas everywhere & pulmonary ecchymoses.
Puppy				
,,				All survived, no symptoms.
,,				
,,				
Dog	9	not stated	1 hr.	Body temp. 20° C. on decompression, gas in heart, dies.
Dog	10	not stated	30′	Paralysed, dies.
Dog	10	not stated	50′	Paralysed, gas in heart, dies.
Dog	10 in $1\frac{1}{2}$ hr.	10′	Rapid decomp. for each 2 atm. then pause for 15′. In all 70′	Slight symptoms in legs, recovers.
Dog	10 in 1 h. 12′	not stated	1 hr. 30′	Slight paralysis of hind limbs, recovers.
Dog	10 in 1 hr.	30′	10—6 atm. in 1′ 6—1 ,, in 1 hr.	Temporary paralysis of hind limbs, recovers.
Dog	10	not stated	10—$4\frac{1}{2}$ atm. in 56′ $4\frac{1}{2}$—1 ,, in 3′	Paralysed, gas in heart, dies.
Dog	10	7′	10—6 atm. in 2′ then 30′ pause, 6—3 atm. in 2′ then 45′ pause, 3—1 atm. in 15′. In all 1 hr. 30′	Survives. No symptoms.

Summary. 11 recovered with no symptoms.
2 died when decompression lasted 1 hr. (in both these the temp. had fallen owing to oxygen poisoning).
1 died when decompression lasted 50′.
5 died when decompression lasted less than 30′.

Seven rabbits at $6\frac{1}{2}$—8 atm. survived rapid decompression.

The most striking of Bert's results is the following : A dog was put at $9\frac{1}{2}$ atm. The apparatus burst. The dog instantly died. Enormous subcutaneous emphysema was found, with gas in stomach, omentum, anterior chamber of eye, cerebro-spinal fluid and spinal cord. The right heart was full of gas which on analysis yielded $15\cdot2$ % CO_2, $82\cdot8$ % N_2, and $2\cdot0$ % O_2.

Having observed the effect of rapid decompression, Bert found that dogs may be safely exposed to $+10$ atm. if 1—$1\frac{1}{2}$ hours be taken for decompression. The animals must of course not be exposed too long, or oxygen poisoning will result.

G. Thompson[1] compressed monkeys, cats, dogs, and pigeons to $+4$— $4\frac{1}{2}$ atm. of oxygen or air for $1\frac{1}{2}$ hours : there were no symptoms unless the decompression were too rapid. A dog stood a presssure of $+8$ atm. for some time without discomfort. He then had a slight convulsion, but was all right after decompression. Catsaras[2] decompressed a dog in 30 seconds after exposure to 5 atm. for 30 minutes. The dog became paralysed in the left leg. Necrobiosis in the left lateral column of the cord in the mid-dorsal region and descending degeneration were found $2\frac{1}{2}$ months later.

Hersent exposed 7 dogs to $+5$ atm. for a few hours and decompressed them in 1 hour to 1 hour 15 minutes.

No symptoms resulted eighteen times. Slight paralysis of the legs occurred 4 times. Paraplegia, cured in 5 days, occurred once. Death with gas in the heart resulted once. One dog decompressed in 50 seconds died. Two dogs decompressed in 15 minutes had paraplegia, and 1 died.

Hersent also exposed dogs to $+3$—$3\cdot8$ atm. for times varying from 11 minutes to 4 hours 11 minutes. The decompression lasted only 30 seconds to 50 seconds.

Out of 9 exposures, there occurred 2 cases of severe paralysis and death with bubbles in the spinal vessels, and 2 cases of temporary paralysis of the legs. All these experiments show the frightful risks of rapid decompression and that even 1 hour for decompression is not quite sufficient for a 4 hours shift at $+5$ atm.

Hersent[3] exposed a man to $+5\cdot4$ atm. for 1 hour and decompressed him in 3 hours. Itching and 'bends' were not prevented, but no serious illness resulted.

[1] *New York Med. Record*, 1889.
[2] *Arch. de Neurologie*, 1890, p. 48. [3] Cit. after Snell, p. 149.

The following preliminary experiments were carried out by one of us (L. H.).

(1) Two large toads compressed for 1 hour in 20 atm. O_2 and rapidly decompressed. The animals went into tetanic spasms, and swelled to double their size with the gas set free in their tissues. The heart was enormously distended, tense and scarlet in colour. On letting out the froth it began to beat vigorously.

(2) Toad in 20 atm. O_2 for 5 minutes. Decompressed in 1 minute. There was some temporary paralysis of the legs and inertness. The animal soon recovered and hopped away into a corner. The same toad was placed in 20 atm. O_2 for 35 minutes. After rapid decompression it was found alive, breathing and trying to escape. In about 1 minute there followed tetanic spasms and rigidity of the legs. The heart was enormously distended, immobile, scarlet and tense. On letting out the froth it began to beat. Gas bubbles were seen in the walls of the intestine, in the lymph spaces, in the anterior chamber of the eye, in the pial vessels etc. The lungs were enormously distended. The nerves and muscles were excitable, and the muscles contracted vigorously.

(3) Rat raised to 15 atm. O_2 in 4 minutes, then rapidly decompressed. The rat violently cleaned its face, there was tremor and tendency to spasm. The animal remained dull and inert but recovered next day.

(4) Rat in 20 atm. O_2 for 6 minutes. Rapid decompression. Respiration almost failed, tendency to tetanic spasms, paralysis of hind legs, contracted pupils, died in 80 minutes. Air bubbles were found in the liver, mesenteric vessels, numberless small ones in the mesenteric fat in the uterus and foetal membranes (the rat was pregnant). The spleen and intestines were greatly congested. There was almost no blood in the heart. No naked eye haemorrhages in the central nervous system.

(5) Rat to 20 atm. O_2 in 5 minutes. Decompression to 7 atm. in 10 minutes and to O rapidly. Immediate convulsions, eyeball projecting, retinal haemorrhages seen with ophthalmoscope, died in 10 minutes. Froth in the heart, and bubbles in the intestinal vessels and walls. No naked eye haemorrhages in central nervous system.

(6) Rat in 20 atm. O_2 for 2 minutes. Decompression in 1 minute. Rapidly cleaned face, dazed condition, a touch caused a violent jump like in first stage of strychnine poisoning. Recovered.

(7) Rat in 20 atm. O_2 for 5 minutes. Rapid decompression. Lay on its side partly paralysed. Soon recovered and moved into cage. On pulling it out it struggled, and this caused a violent epileptic fit (due to displacement of gas bubbles by the struggling?). It quickly recovered and ran into the cage.

(8) Rat in 20 atm. O_2 for 9 minutes. Rapid decompression. Collapse paralysis, gasping respiration and death. Air bubbles everywhere in the right heart, liver, stomach and mesenteric vessels. General emphysema of the fat and connective tissues.

(9) Guinea-pig in 10 atm. O_2 for 2 minutes. Rapid decompression. The animal at first appeared dazed but soon recovered.

(10) Guinea-pig to 22 atm. O_2 in 4 minutes. Rapid decompression. Convulsions, rolling over to right, death. Froth in the heart and lungs. Some air bubbles in wall of intestine. Small pin point haemorrhages over base of brain. A few bubbles in the larger pial vessels.

The microscopical examination of the organs of these animals was carried out by Dr Finlayson. In the central nervous system the gas-bubbles formed small cyst-like cavities surrounded by compressed and flattened nerve-cells. The same kind of cavities formed in the liver. The bubbles set free in the vessels run together at less resistant points and the vessels become alternately occupied with columns of corpuscles and long bubbles of gas. Bubbles are also set free in all the connective tissue (lymph) spaces throughout the body and especially in adipose tissue. We have never seen bubbles actually within a muscle-fibre, nerve or other cell. The cells are not torn but compressed and rendered anaemic. It is easy to conceive how the escape of gas-bubbles into resistant structures such as bone, aponeurosis nerve-roots and nerve-sheaths may give rise to the 'bends' or pains so commonly suffered by caissoniers. Von Schrötter has published a figure exhibiting the air-bubbles in the coronary artery of a dog decompressed rapidly from 4 atm. He also gives a figure of the lesions produced in the spinal cord by the air-bubbles[1].

We have actually observed the production of air embolism in the vessels of the frog's web and bat's wing. The animals were exposed to 20 atm. for about 10 minutes. For about a minute after rapid decompression the circulation continued unaltered, then small dark bubbles were seen, first one, and then another, and then numbers scurrying through the vessels, and driving the corpuscles before them. In a moment or two the vessels became entirely occupied with columns of air bubbles, and the circulation was at an end. By means of rapid recompression we have driven the gas again into solution, and have seen the corpuscles reappear in the capillaries and the circulation become reestablished. On very *slowly* decompressing the animals we have seen no gas bubbles appear.

Our large pressure chamber, pump and other facilities kindly provided by Messrs Siebe and Gorman, the well known marine engineers, has enabled us to thoroughly study the effects of rapid and slow decompression. The chamber was provided with a large tap, by means of which the pressure could be lowered from + 7 atm. to + 0 in about 10 seconds to 1 minute.

It was also provided with a pin point opening through which the period of decompression could be made to occupy 1, 2 or more hours.

Experiment I. A large cat, two half-grown rabbits, two large rats, and two white mice were placed in the chamber and the pressure raised to 105 lbs. (+ 7 atm.).

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[1] *Prager med. Wochenschrift*, xxiv. 1899, Nr. 14.

A ventilation current was maintained. All the animals appeared to be perfectly normal. At the end of an hour rapid decompression was brought about. The chamber filled with mist owing to the cooling of the expanded air. When the mist cleared we saw that the cat and one rabbit were dead, while the other rabbit was in violent tetanic convulsions.

On opening the chamber the rats were found to be dead.

The second rabbit died also and the mice alone survived.

There was emphysema of all the tissues and frothing of the blood in the right heart and lung. In the albino rats we could see extensive retinal haemorrhages.

II. A cat was placed in the chamber and the pressure raised to +7 atm. in 50 minutes, and then rapidly lowered to +0. The cat survived, for the tissue fluids had not become sufficiently saturated with air.

III. A large cat, a rabbit, two white rats and two mice were compressed to +7 atm. in 50 minutes, and kept at this pressure for 1 hour. Decompression occupied 1 hour. None of the animals showed any discomfort.

IV. A Rhesus monkey, a rat and 2 mice were compressed to +7 atm. for 4 hours. The animals seemed untroubled by the pressure. Decompression was started at 4·30 p.m. by opening the small tap ; the last part of the decompression was hastened and when at 5·25 the pressure registered 10 lbs. to the sq. inch, the large valve was opened and the pressure quickly brought to zero. On opening the chamber the monkey and the other animals seemed perfectly normal. On removing the monkey from the chamber he struggled to escape but in the course of a minute or two suddenly became quiet and lay on his side gasping, and with a peculiar cry. He gradually got more and more dyspnoeic, and his lips, tongue and face became markedly cyanotic. Despite energetic artificial respiration he died in about 10 minutes after removal from the chamber.

Post mortem. Heart : not markedly distended, ventricles in 'delirium cordis,' auricles beating feebly. On opening the right heart a little deep purple frothy blood exuded followed soon however by non-frothy blood. *Mesenteric veins* : small air columns in several of these. *Lungs* perfectly healthy.

The other animals in this experiment did not show any decompression symptoms. The cause of the trouble was no doubt the acceleration of the last part of the decompression.

V. The experiment was repeated with another monkey (Rhesus). After being subjected to +7 atm. air for 4 hours, 2½ hours were taken to decompress. There was not the slightest sign of decompression symptoms.

This experiment was repeated on this monkey three or four times a week for a month, the time for decompression being in each case 2 hours. There was never the slightest sign of decompression symptoms and the monkey remained in perfect health and maintained its weight. Towards the end of the period of compression it sometimes seemed to become sleepy. The body temperature remained normal.

The treatment of the decompression symptoms.

As we have seen in the experiments on the frog and bat the bubbles of air, which develop in the capillaries, pass back into solution on a rapid reapplication of the pressure.

We have tried this in the case of larger animals.

Experiment VI.

A large hutch rabbit was kept under a pressure of +7 atmospheres of air for 4 hours and was then quickly decompressed. In a minute or so the rabbit developed typical decompression symptoms (i.e. fell on side and limbs showed tetanic convulsions). The pressure was now quickly re-applied up to about +5 atm. by emptying a large cylinder of compressed air into the chamber. The symptoms however remained unabated and the rabbit soon died. It was evident, therefore, that for the re-application of pressure to be of any avail, the pressure must be very quickly re-established and no time be given for the air bubbles to tear up and damage permanently the nervous tissues, or to produce stasis of the circulation for too long a period.

We, therefore, repeated the experiment with the modification that the pressure was more quickly re-applied.

VII. A cat and a hutch rabbit were subjected to an air pressure of +7 atm. for 4 hours. Decompression was effected to zero in about five seconds, and as quickly as the taps could be opened (about five seconds) a large cylinder of compressed air was delivered into the chamber, thus raising the pressure to 95 lbs. in about 2 minutes.

At the moment of decompression the cat sprang to the window, excited and with widely dilated pupils. In a few seconds it became entirely paralysed in the limbs so that it fell helpless on to its side, its head meanwhile showed continuous side to side pendulum-like movements. There was no mystagmus. On recompression, these symptoms gradually disappeared, the head movement being the first to go, then the pupils contracted to their normal size. Some two or three minutes after recompression to 95 lbs. the cat tried to move about but fell. The pressure was maintained for 45 minutes and then slowly lowered. The cat recovered and on removal seemed *perfectly normal*, and on being placed in his basket leapt over its side and escaped into the room. Next morning it was quite normal in every respect.

The rabbit was recompressed before it showed any symptoms of decompression and was quite normal on removal from the chamber.

All our other experiments on metabolism, oxygen poisoning etc. show that for +7 atm. 2—3 hours is a safe period for decompression. The only case in which it fails is when the animals have developed symptoms of oxygen poisoning and have become comatose, their body temperature lowered and lungs congested by too long a stay in the compressed air. The circulatory and respiratory organs then fail to rid the body of the gas with which it is saturated.

Our experiments confirm those of Bert.

The blood and tissue juices effervesce on rapid decompression like an opened bottle of soda water. The longer the shift the greater becomes the saturation of the body fluids, and the greater the risk of rapid decompression.

A 5 minutes exposure to $+20$ atm. O_2 is sufficient to saturate rats and guinea-pigs so far that they die on rapid decompression.

Animals can be exposed to $+7$ atm. air with perfect safety for four hours, and be brought out quite well when the period of decompression is made to last 2 hours.

Recompression, after rapid decompression, causes solution of the gas, and may, if quickly applied, save the life of the animal. Recompression has been found to alleviate the bends in most caisson works, and Mr Moir introduced a boiler at the Hudson tunnel wherein recompression was applied with excellent results. At the Blackwall tunnel a 'medical lock' was likewise employed and the cases of bends frequently derived benefit from recompression followed by slow decompression. Von Schrötter, from his experience at Vienna caissons, considers recompression to be the sovereign remedy for caisson sickness if it can only be applied in time.

We will now contrast the experimental results with the periods of decompression employed at some of the chief caisson works, and then discuss the influence of age and habit of body on caisson illness.

Periods of shift and decompression at Caisson works.

Atm. (maximal)	Length of shift	Period of decompression	Place
$4\frac{1}{4}$	4 hrs.	30'	Chalonnes
2	—	10"	Lorient
$3\frac{1}{2}$	4 hrs.	12—15'	Kehl
		(rule often broken by men)	
$3\frac{1}{2}$	—	20'	Trazegnies
3	8 hrs.	4—5'	St Louis
$3\frac{1}{2}$	4 hrs.	10'	,,
4	3 hrs.	18'	,,
2—3	8 hrs.	4'	Blackwall
		(often shortened to 30" by men breaking the rules)	

Triger recommended 7 minutes.

Barella recommended 10 minutes per atm.

Foley recommended 3 minutes, and considered slow decompression dangerous.

The Greek divers are usually pulled up rapidly.

Denayreuze for divers recommended 1 minute per metre.

Siebe and Gorman recommend deep divers to take 20 minutes in ascending.

Paul Bert recommended short shifts and 30 minutes decompression for 2—3 atm. and 60 minutes decompression for 3—4 atm. The decompression chamber must, he says, be warmed.

For deep divers he recommends a half-way resting stage.

Catsaras recommends a rest of 1 minute at every two fathoms of ascent. He also advises sponge divers not to stay longer than 5 minutes at 25 fathoms, and only 1 minute at 30 fathoms.

Influence of Age. Pol and Watelle state that young men of 18—26 years stand the work best; out of 25 men discharged on account of symptoms 19 were over 40 years old.

E. H. Snell found that at the Blackwall tunnel men below 20 were immune to accidents of decompression. This agrees with the general tenour of experiments on animals. The young bear rapid decompression best. He publishes the following table.

Age	No. of men passed	No. of cases taken ill whose ages are recorded	% illness
15—20	55	0	0
20—25	145	15	10·3
25—30	152	37	24·3
30—35	91	19	20·9
35—40	61	14	22·9
41—45	38	10	26·3
45—50	3	5	166

Habit of body. In stout men or men of heavy build the liability to illness is greatly increased. A. Smith compiled the following table from the records, at Brooklyn bridge, of men under 45 years.

	Spare	Medium	Heavy
Lost little or no time from sickness	25	14	3
Taken sick	28	22	36
Paralysed	2	3	8
Died	—	—	3

Considering that under 45 years heavy men are greatly in the minority, this report is most striking. Snell excluded old and heavy men from the Blackwall tunnel caissons, and lost no cases. Men prematurely grey and with commencing arterial degeneration should also be excluded.

There is no proof that long continuance at the work renders a man immune. Cases frequently occur among old hands. The men among the new hands who are liable to attack are discharged. Divers who have when young done deep jobs become paralysed at less depths in advanced years.

A. Smith says that severe exertion after decompression predisposes to attack. This is to be expected, for the exertion may force the air bubbles in the blood vessels out of harmless into harmful places. We have brought on attacks of convulsion by massaging the abdomen of rapidly decompressed animals. The monkey in experiment IV. died after struggling.

Ventilation. E. H. Snell lays great stress on the good results which follow free ventilation of caissons. He says, "An increase of CO_2 from ·04 % to ·1 % at 30 lbs. pressure is the forerunner of much illness."

In one of the Blackwall caissons where the pressure was 25—35 lbs. illnesses were occurring at the rate of seven a day. The men were working at the bottom of the caisson; the air supply pipe opened near the roof and the air escaped again through the roof. The supply pipe being lengthened, the illnesses at once dropped to an average of 1 in 2 days.

The following table has been compiled by Snell to illustrate the effect of increased ventilation.

Caisson I. Pressure $+25$—35 lbs.

Cubic feet of air per man per hour	No. of days	Cases of illness	Illnesses per 100 days
Below 4000	13	41	315·5
4000—8000	26	78	300
8000—12000	10	8	80
Above 12000	12	4*	33·3

* Only 2 or 3 men were in the caisson on the days when these illnesses occurred, and so the total volume of air supplied to the caisson was reduced, i.e. the air supplied per man was high, but low per caisson.

In other tables Snell seeks to prove that a ventilation of over 12,000 c. ft. per man abolishes illness. He points out that candles smoke in compressed air, but cease to do so if put within a lamp chimney so as to increase the draught. As the velocity of diffusion of a gas varies inversely as the sq. root of the density he attributes the smokiness of the candle to the slow diffusion of the products of combustion. He thinks the same may hold good for man.

Snell suggests that an increase of CO_2 from ·04 to ·1 % may actually be the cause of caisson illness and that CO_2 may be the gas which in particular is set free in the blood on decompression. There is no evidence that this amount has the slightest toxic effect.

At Brooklyn caisson with ·33 % CO_2 was found on analysis, which at 3 atm. gives ·99 % atm. CO_2. At Blackwall with ·1 % CO_2 and at Brooklyn with ·33 % CO_2 the extra CO_2 would cause a slightly increased

depth of breathing and thus practically no effect on the composition of
the alveolar air in contact with the blood. This, at least, would seem to
follow from experiments recently communicated to the Physiological
Society by Haldane and Priestley. One of us (L. H.) recently measured
the CO_2 in the chamber when a cat was exposed to 8 atm. of air.
There was finally as much as 1%, i.e. 8% atm. The cat breathed
more deeply but otherwise did not show any ill effect.

We cannot suppose that a small percentage of CO_2 in the air would
contribute in any way to the setting free of CO_2 bubbles on decom-
pression. It is true that Paul Bert found $15\% \ CO_2$ and $85\% \ N$ in the air
obtained from the heart of animals killed by rapid decompression. When
blood is exposed to air it gives off CO_2 owing to the very low partial
pressure of this gas in the atmosphere. Similarly when nitrogen gas is
set free in the heart, some CO_2 will diffuse out, and Bert found some
traces of oxygen. Bert's analyses of blood gases show that the CO_2 in
the arterial blood is if anything lessened under the influence of com-
pressed air, and our results confirm Bert.

Hunter notes that the most dangerous times in the Forth Bridge
caisson were (1) when soft wet silt was being removed, (2) when con-
creting was going on. It must be borne in mind that the presence of
traces of a toxic gas such as H_2S is dangerous in compressed air, owing
to the increase in the partial pressure of such impurities. In the case
of CO the increase in partial pressure will be balanced by that of
oxygen. But the CO might produce its effect on decompression. The
caissons become fouled with the excretions of the workmen, and it is
very needful that proper earth-pails should be provided.

As matters stand at present it is not easy to explain Snell's
ventilation results, and it is urgently required that they should be
confirmed.

Increased rate of ventilation has not seemed to affect our animals in
regard to the dangers of decompression.

From the records of caisson sickness and from our experimental
results we conclude that the men selected for high pressure work should
be small men, of spare and wiry habit, not older than 20—25. The
men should be total abstainers and abstemious in all their habits.

The men should all be tested at low pressures first, and those who
suffer from symptoms should be discharged.

The following we consider to be safe rules for working.

The longer the shift, the greater is the saturation of the body fluids
with gas, and the slower therefore should be the decompression.

The higher the pressure, the shorter should be the shift, and the longer the decompression.

We suggest the following times as safe:

Atm.	lbs.	Shift	Decompression period
+2	30	4 hrs.	30'—1 hr.
+3—4	45—60	4 hrs.	1—2 hrs.
+5	75	1 hr.	1—2 hrs.
+6—7	90—105	30'—1 hr.	2 hrs.

To prevent men breaking the rules the decompression chamber should be provided with one cock only, which will allow decompression to take place in the given time. A separate lock should be provided for the rapid passage of material. The decompression chamber must be artificially warmed so that the temperature does not fall below 60° F., and it must be thoroughly ventilated during decompression.

The ventilation of the caisson or diving apparatus should be very free, and the temperature of the air should be about 60° F. The men should remain quiet for an hour or so after decompression and be recompressed on any sign of sickness. Paul Bert recommends that oxygen be supplied to the decompression chamber in order to hasten the diffusion of nitrogen. This is no doubt a means by which the period of decompression might be shortened, but it introduces the danger of oxygen poisoning.

We are of opinion that by proper choice of men and regulation of the shift and decompression period, work could be carried out without loss of life at a depth of even 200 ft., i.e. about 7 atm. or + 100 lbs. pressure.

Summary.

1. Compressed air above 5 atm. lessens the CO_2 output, and lowers the body temperature in mice, rats, and young rabbits.

2. Oxygen at and above 1 atm. has the same effect. It is a sign of oxygen poisoning.

3. Compressed air at 10 atm. is more damaging—at least to small animals—than oxygen at 2 atm.

4. Compressed air increases the loss of body heat both because it is a better conductor, and because it is saturated with moisture.

5. The saturation of the air with moisture in caissons does not prevent evaporation from the body because the skin temperature is above that of the air. The wet air by damping fur or clothes increases loss of heat.

6. Highly compressed air may possibly interfere with the diffusion

of CO_2 from the alveolar air, and may, owing to increased friction, hinder the passage of air in and out of the air-tubes.

7.   The nitrogen output in dogs is not altered in any noteworthy degree by exposure for six hours to 8 atm. air.

8.   Inflammation and consolidation of the lungs is produced by exposure to 8 atm. air for over 24 hours. $1\frac{1}{2}$ atm. of pure oxygen has a similar effect.   The higher the oxygen tension the more rapidly does the inflammation ensue, e.g. 6 atm. O_2 produces marked congestion in 2 hours.

9.   It does not seem likely that inflammation of the lungs should be produced in the pressures and times of exposure usual in caissons.

10.   Excised frog's hearts, muscles, and nerves are not rapidly poisoned by even 50 atm. O_2.   A heart will beat more than an hour exposed to this pressure.   The vagus nerve endings appear to be paralysed by such exposure, while inhibition can be obtained by stimulating the crescent.   The thin sartorius muscle is much more easily affected than the gastrocnemius, and soon gives a curve like a fatigue curve.

11.   All animals investigated, vertebrates and invertebrates, are instantly convulsed and killed by exposure to 50 atm. O_2.

12.   Convulsions are frequently produced in vertebrates by exposure to 4—5 atm. O_2, while exposure to 6—25 atm. O_2 produces dyspnoea and coma, and the convulsive stage does not usually appear.   Cleaning movements, salivation, gaping, jerky deep respiration, are symptoms which precede the convulsions, and coma soon follows them.

13.   We have not observed convulsions with air pressures up to 12 atm.   Salivation, dyspnoea, and coma are the symptoms.

14.   The blood-gases increase in compressed air or oxygen according to Dalton's law, but the process of complete saturation of blood and tissues takes some time.

15.   The circulation is unaffected mechanically by compressed air.

16.   The cause of caisson-sickness is the escape of gas bubbles in the blood vessels and tissue fluids on decompression.   An animal exposed for 4 hours to 8 atm. air and quickly decompressed is like an opened bottle of soda-water.   The fluids of the body generally effervesce.

17.   The effervescence can be studied in the circulation of the frog's web or bat's wing, the animals being enclosed in a suitable chamber. It takes a little time for the bubbles to grow to an appreciable size.

18.   Recompression causes the bubbles to go into solution, and if applied quickly enough the circulation recommences.

19. The bubbles after rapid decompression can be seen post-mortem in the blood vessels, in the heart, retinae, aqueous humour, connective tissue spaces, etc. The alimentary canal is blown out with gas. The bubbles produce cyst-like cavities in solid organs, e.g. in the central nervous system, the liver. The cells are compressed round these cysts.

20. In the case of oxygen an animal may recover after an extraordinary amount of this gas has been set free by rapid decompression. The nerve cells are not killed by the oxygen bubbles, and the animals are convulsed and exhibit hyper-reflex-excitability.

21. The varying symptoms of caisson-sickness are due to the varying seat of the air emboli.

22. Young men escape caisson-sickness owing to the elasticity of their tissues, and greater facility for collateral pathways of circulation.

23. Animals can be safely exposed to 8 atm. of air for 4 hours if 2 hours be spent in gradual decompression. Such exposure can be safely repeated three times a week.

24. By the choice of suitable men, and proper regulation of the period of compression and decompression, caisson and diver's sickness can be avoided.

PUBLIC HEALTH AUTHORITIES IN RELATION TO THE STRUGGLE AGAINST TUBERCULOSIS IN ENGLAND.

By ARTHUR NEWSHOLME, M.D., F.R.C.P.,

Medical Officer of Health of Brighton[1].

THE task with which I have been honoured—of reporting upon the action of English Public Health Authorities in regard to tuberculosis —can, I believe, be best fulfilled (*a*) by a review of the history of the mortality from tuberculosis in England since 1837, when vital statistics first became available, (*b*) by a statement of the factors which have been instrumental in causing the reduction in the mortality from tuberculosis shown in these statistics, and an attempt at weighing their relative importance, (*c*) by a description of the more direct measures taken in England to diminish the prevalence of tuberculosis, and (*d*) by a forecast of the lines on which preventive measures against this disease are likely to be extended.

(*a*)   *Review of the Mortality from Tuberculosis in England.*

In 1838–42, the five first complete years of registration of deaths in England, the recorded death-rate from phthisis averaged 3·88 per 1000 of population (see Table IV.).   It is doubtful if this figure can be trusted.   At this time medical certification of the cause of death was not compulsory, and did not become so until 1874.   Even in 1871 in about 8 per cent. of the deaths the cause was not certified by a qualified practitioner, though there had been a steady decrease in the proportion of uncertified deaths.   Before this many deaths were probably ascribed to "consumption" on the strength of insufficient non-medical evidence. From the fifth decade onwards the statistics have become more trustworthy, subject to the further remarks on page 448.

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[1] A paper read at the Meeting of the International Congress of Hygiene and Demography, Brussels, Sept. 1903.

The main results are given in the following table :—

TABLE I.

Death-rate from Phthisis Pulmonalis and from other Tubercular Diseases in England and Wales per million living of each sex.

Period	Phthisis		Other Tubercular Diseases	
	Males	Females	Males	Females
1851—60	2579	2774	915	700
1861—70	2467	2483	870	665
1871—80	2209	2028	849	651
1881—90	1847	1609	775	620
1891—95	1633	1303	658	
1896—1900	1321		581	

From the year 1851 to 1865 the phthisis death-rate was greater among females than among males, the difference between the two gradually diminishing. Since 1866 the phthisis death-rate has been uniformly in excess among males and increasingly so. The comparative male and female death-rates from phthisis and from other tubercular diseases during the last twenty years are shown in Table II.

TABLE II.

England and Wales. Annual Death-rate per million living from

Year	Phthisis		Other Tubercular Diseases	
	Males	Females	Males	Females
1881	1920	1735	794	621
82	1947	1758	824	643
83	1967	1797	787	639
84	1927	1733	823	669
85	1875	1670	736	589
86	1874	1612	821	652
87	1728	1508	649	591
88	1717	1428	724	586
89	1719	1435	755	621
1890	1868	1506	775	618
91	1780	1429	782	626
92	1624	1321	735	603
93	1635	1304	542	612
94	1559	1215	643	532
95	1559	1237	738	592
96	1480	1133	646	524
97	1526	1155	650	527
98	1509	1123	659	537
99	1549	1123	632	517
1900	1570	1110	630	510

Between 1881 and 1900 the male phthisical death-rate has declined 18·2 per cent., the female phthisical death-rate 36 per cent. The decline in other tubercular diseases in the same period is for males 20·6, for females 17·9 per cent.

The decline in the death-rate from phthisis has not been uniform in extent at different ages. In the following table I have calculated the percentage reduction of the phthisis death-rate at each age-group for the two sexes, the comparison being between the average death-rates for 1851–60 and for 1891–95.

TABLE III.

Percentage Reduction of Phthisis Death-rate between 1851–60 *and* 1891–95.

	All ages	0—	5—	10—	15—	20—	25—	35—	45—	55—	65—	75 and upwards
Males	37	65	63	66	55	50	37	18	16	19	34	39
Females	53	57	58	57	59	59	53	45	44	46	51	51

Thus the decline has been greatest at ages under 20 in males and under 25 in females. The greater decline at the lower ages and in the female sex may be regarded as indicating that the domestic causes of tuberculosis have been removed to a greater extent than the occupational, to which men are particularly exposed. This is probably the case; but there is the disturbing fact that registered deaths from phthisis now more closely represent the real facts as to this disease than they did in the earlier years of registration of causes of death. The term phthisis or consumption is not now used so loosely as formerly, when any chronic chest ailment accompanied by wasting was liable to be called by this name. In recent years, furthermore, there has been an increasing practice on the part of medical practitioners to return deaths as due to " tuberculosis," which would formerly have been returned as phthisis. The result has been some exaggeration of the decline of the death-rate from pulmonary phthisis.

The statistics as to "other tubercular diseases" given in Tables I. and II. cannot be regarded as trustworthy. They include tabes mesenterica, tubercular meningitis, and other forms of tuberculosis (scrofula, etc.). In many instances in which the diagnosis of " tabes mesenterica " has been made, it has been shown post-mortem that there is no evidence of tuberculosis in the abdominal cavity.

When however allowance has been made for the errors of certification and registration to which reference has been made, it remains clear that there has been a most gratifying decline in the mortality from tuberculosis.

The amount of registered decline in each successive period of five years is shown in Table IV.

TABLE IV.

Phthisis Mortality.—Persons.

Period	Death-rate per Million of Population	Percentage Decline in Mortality from Phthisis in each Period as compared with that in the immediately preceding Period
5 years 1838—42	3880	
No statistics 1843—49		
5 years 1851—55	2851	32·9[1]
,, 1856—60	2603	8·7
,, 1861—65	2528	2·9
,, 1866—70	2448	3·2
,, 1871—75	2218	9·4
,, 1876—80	2040	8·0
,, 1881—85	1830	10·3
,, 1886—90	1635	10·7
,, 1891—95	1461	10·7
,, 1896—1900	1321	9·6

If, as I believe is the case, the extremely high registered death-rate from phthisis in 1838–42 may be ignored as untrustworthy, and if, as is probable, for similar reasons the apparent decline between 1851–55 and 1856–60 is greater than the real decline, we then have remaining a series of years which can be divided into two groups. The first comprises the decennium 1861–70, in each quinquennium of which the decline was about 3 per cent.; and the second comprises the years after 1870, in which the decline has averaged from 8 to 10 per cent. The transition from the first to the second of these periods is abrupt.

(b) *Causes of the Diminution in Mortality from Tuberculosis in England.*

Considerations of space oblige me to assume a knowledge of the general course of sanitary legislation and administration in England. It is important to note that (excluding the doubtful years mentioned above) in the years in which sanitary administration first became

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[1] This represents the decline of 13 not of 5 years. Reduced in the proportion of 13 to 5 it becomes 12·7 per cent.

generally operative throughout the country (1871–75) the decline in mortality from phthisis was triple that in the preceding quinquennium, and the higher rate of decline has continued up to the present time. It is not necessary to apportion the share in this decline which is to be attributed to works of main sewerage, to better systems of removal of domestic excretal and other refuse, to drying of the sub-soil[1], to diminished crowding in houses and their improved cleanliness, to improved industrial conditions caused by the operation of the Factories and Workshops and other Acts, and to the effect of the Compulsory Education Act, 1870. All of these have borne their part in producing the diminished mortality from tuberculosis. They almost certainly are not the only factors concerned, and the means by which the abolition of some of the above factors has caused a reduction of tuberculosis has probably been in part misinterpreted. Thus some of the most important of the above measures have directly diminished the opportunities for infection. Improvements in housing and the diminution of overcrowding undoubtedly have had this effect. The improved habits of the people have had the same effect to an even greater extent. Quite apart from the present crusade against spitting, there has been an immense improvement in the national habits in this respect and in general domestic cleanliness, which must have had a material effect in diminishing opportunities for infection. At the same time I do not wish to minimise the importance of indirect measures against tuberculosis. Opinions will doubtless differ as to the relative share which direct infection by means of spray during coughing or by dried expectoration on the one hand, and dirty, overcrowded, ill-lighted dwellings on the other hand, play in the causation of tuberculosis. All who wish to effect the most good will endeavour to the utmost to control both sets of factors.

Sir Hugh Beevor[2] has emphasised the importance of abundant food in the prevention of tuberculosis. He shows "a coincidence and a remarkable agreement between the fall in the phthisis-rate, the number of paupers, and the rise in the average wage." The Corn Laws prohibiting the free importation of corn into England were abolished in 1846, taking full effect on February 1, 1849, after which day the duty on imported corn became 1s. per quarter only. The 1s. duty was abolished

[1] In the *Practitioner*, New Series, Vol. XIII. 1901, page 206, I have given my reasons for regarding wetness of soil as of relatively small importance in the causation of tuberculosis.

[2] *Hunterian Oration*, 1899.

by an Act passed on June 24, 1869. The average price of wheat in the ten years 1837–46 was 56s. 7d., in 1860 it had become 53s. 3d., in 1862 55s. 5d., since when it has declined to a minimum of 22s. 10d. in 1894 (see Table V. and Figure). It should be noted that owing to the failure of the potato crop, the Crimean War, and the depreciation of gold, the price of corn, notwithstanding unrestricted importation, did not decline until 1862[1].

TABLE V.

Average Prices of Wheat and Phthisis Mortalities.

Years	Price of Wheat per quarter in pence[2]	Death-rate per million of population from Phthisis	Proportional amounts in each Quinquennium. Average for entire period = 100	
			Wheat	Phthisis
5 years 1838—42	775·6	3880	141	172

No statistics as to the cause of death were kept 1843–49 inclusive.

Years	Price of Wheat per quarter in pence	Death-rate per million of population from Phthisis	Wheat	Phthisis
5 years 1851—55	668·6	2851	121	126
,, 1856—60	640·0	2603	116	115
,, 1861—65	568·0	2528	103	112
,, 1866—70	655·6	2448	119	108
,, 1871—75	655·6	2218	119	98
,, 1876—80	570·0	2040	103	91
,, 1881—85	481·2	1830	87	81
,, 1886—90	376·8	1635	68	72
,, 1891—95	334·8	1461	61	65
,, 1896—1900	343·0	1321	62	59

The close coincidence between the price of wheat and the phthisis death-rate is shown more clearly in Fig. 1. The reasons for this coincidence are not far to seek. The more abundant and cheaper supply of food has doubtless improved the physical condition of the population and made them less susceptible to the inroads of tuberculosis. It is not however simply and solely a question of cheaper food. The English population as a whole has increased its standard of comfort and has been able to afford not only better, more abundant, more varied, and more wholesome food, but is also better clad and better housed than it has been in the past. In 1861 a penny on the income-tax yielded £1,100,000, in 1901 it yielded £2,400,000, an increase of 118 per cent., while the population of Great Britain only increased 43½ per cent. in the interval. This improvement has affected not only those who pay income-

[1] Giffen: "Progress of the Working Classes, etc." *Journ. Statist. Soc.* 1883–4.
[2] *Journ. Statist. Soc.* Vol. VIII. Part II. page 254.

tax. Sir R. Giffen, F.R.S., on whose authority[1] the above financial state-
ments are made, showed[2] that while the workman's wages have advanced
(in many trades he obtains from 50 to 100 per cent. more money than 50

Fig. 1.

····· Proportional Price of Wheat　　·—· Proportional Mortality from Phthisis
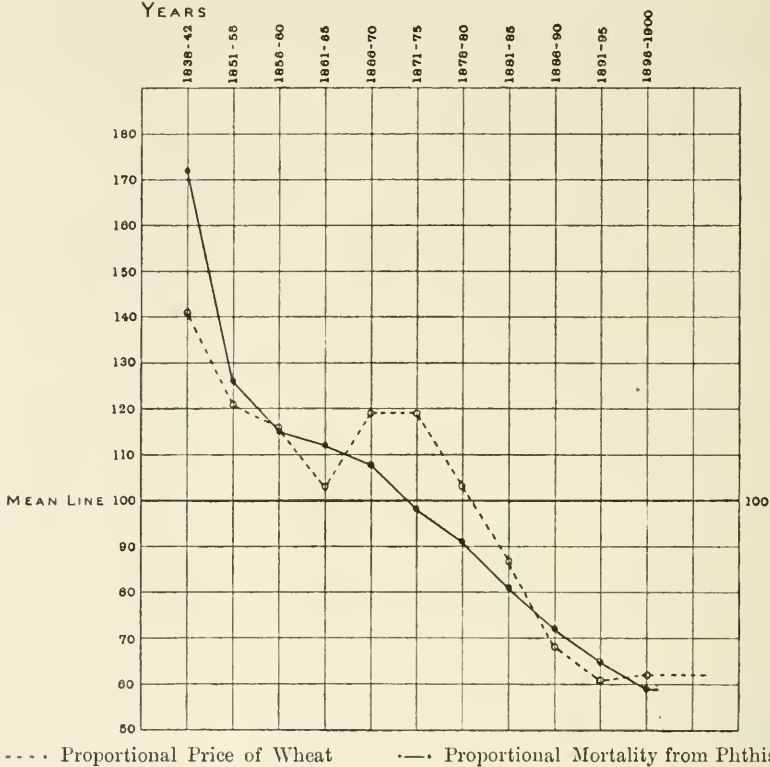
years previously for 20 per cent. less work), most articles he consumes
have diminished in price[3]. While recognising that a section of the
population still lives in extreme poverty, it is I think certain that the
main mass of the English population lives in greater comfort than fifty

[1] "A Financial Retrospect 1861–1901." *Journ. Statist. Soc.* Mar. 1902.

[2] "The Progress of the Working Classes in the last Half-Century." *Journ. Statist.
Soc.* 1883–84.

[3] Mr A. L. Bowley, M.A., after an independent investigation of a large number of trades
concludes that "average income and average wages have increased at nearly equal average
rates, and that both have nearly doubled during the period under review," 1860 to 1891.
(*Journ. Statist. Soc.* Vol. LVIII. Part II. p. 251.)

years ago, and that this has been an important factor in causing the reduction in the mortality from tuberculosis. Poverty and tuberculosis are close companions, for poverty not only furnishes the appropriate soil, but also increases the closeness of contact and the frequency of opportunities for infection, and thus in two ways increases the mischief caused by the tubercle bacillus.

(c) *English Administrative Measures against Tuberculosis.*

This part of my subject may be considered in accordance with the following scheme of preventive measures, which although not exhaustive includes the most important measures.

A. *Means of ascertaining the existence of the disease.*
    1. Bacteriological diagnosis.
    2. Notification of cases, voluntary or obligatory.

B. *Direct preventive measures.*
    1. Law against expectoration in places of public resort.
    2. Disinfection and cleanliness.
    3. Sanatoria.
    4. General sanitary improvement.

C. *Education of the public and of patients in the importance of the preceding measures.*

Bacteriological Diagnosis. In the last few years a bacteriological laboratory has more commonly been recognised as an essential part of the preventive machinery in sanitary administration. Many practitioners examine their patients' sputa for themselves; others however have not the necessary time or skill; and some do not even yet realise its importance in the early diagnosis of tuberculosis. In Brighton, facilities for practitioners in this respect were provided in 1897, and in successive years the number of specimens examined has been as follows:—

		Number of specimens of sputum examined for practitioners
14 months	1897—8	21
12 ,,	1899	47
12 ,,	1900	86
12 ,,	1901	125
12 ,,	1902	169
6 ,,	1903	120

Notification of Cases.   In England the obligation is laid upon the medical attendant and upon the householder or nearest relative of the patient to notify to the medical officer of health the fact that the patient under his charge or in his house is suffering from one of the notifiable infectious diseases (scarlet fever, diphtheria, enteric fever, typhus, small pox, cholera, erysipelas).   Other diseases such as chicken pox, measles and whooping cough may be added to the list by any sanitary authority desiring to do so.   The Central Government Authority (Local Government Board) has however until recently declined to permit local sanitary authorities to extend the provisions of the Infectious Diseases (Notification) Act to phthisis, the reason given being that "the Board have hitherto held the view that phthisis is not a disease to which the provisions of the above-mentioned Act could with advantage be applied."   In the absence of such powers of compulsory notification the medical practitioners of Brighton were asked to co-operate in a system of voluntary notification of cases of phthisis.   Similar steps have been taken in Manchester and Sheffield, and the following table shows the relative amount of voluntary notification secured in each of these communities.

Year	Brighton	Manchester	Sheffield
1899	113 [1]	425 [2]	33 [3]
1900	105	1573	585
1901	153	1339	648
1902	224	1260	739
1903	165 ($\frac{1}{2}$ year)	323 ($\frac{1}{4}$ year)	—
Census Population 1901	123,478	543,969	380,717

[1] Entire year.   [2] From Sept. 6th to end of year.   [3] Dec. 7th to end of year.

Note.   *The figures for Brighton and Manchester are the number of new cases notified, for Sheffield the figures give the number of notifications.*

In a few other towns and districts the voluntary notification of phthisis has recently been practised to a certain extent.

The fear has been expressed that the voluntary notification of a case of phthisis might expose the doctor to the risk of accusation of a breach of professional secrecy, as the notification is not imposed as a statutory duty upon him.   This has been felt by some doctors to be a real difficulty.   I have always, when consulted on this point, expressed the opinion that no case should be notified without the consent of the patient; and in actual practice it is found that this difficulty has limited the operation of voluntary notification of phthisis chiefly to patients of the poorer classes, and particularly those treated in connection with the

poor law, or in public hospitals or dispensaries. Among these patients, and the small proportion of patients of a higher class who are notified, I have found that visits by the medical officer of health or his assistant are not unwelcome, and that the patients are usually grateful for the help they receive in having their rooms cleansed and purified, in being supplied with pocket spittoons and Japanese paper handkerchiefs, and especially in being helped to secure sanatorium treatment.

The difficulty however is an important argument in favour of compulsory notification of cases of phthisis to the medical officer of health ; and it is satisfactory to learn that Parliament during the present Session has consented to a local enactment making phthisis compulsorily notifiable in the city of Sheffield. The powers thus recently conferred on Sheffield are set forth in the Appendix (p. 465). It will be observed that the powers are confined to the enforcement of notification by the medical practitioner and to the enforcement of cleansing and disinfection. No powers of compulsory isolation of phthisical patients are conferred, and no responsible person has so far as I am aware ever suggested or is likely to suggest that the granting of such powers is desirable.

Notification, whether voluntary or compulsory, is but a means to an end. It is necessary therefore to examine the action which is taken in regard to notified cases.

Having had over four and a half years' experience in Brighton of the voluntary notification of phthisis, our procedure has become fairly settled, and I am able to give a trustworthy opinion on the degree of benefit obtainable from it.

The procedure adopted is that (1) the notified patient is visited at home, or is interviewed at my office in connection with his proposed removal to the sanatorium for open-air treatment. At this interview exact details are ascertained as to the duration and history of the illness, the possible sources of infection, places of residence during the illness, occupation and work places during the past five years, habits as to spitting, and so on.

(2) The patient's room is cleansed and disinfected when required. This is always done when a change of address occurs, or when the patient is admitted to the sanatorium. These measures are carried out in every instance after the death of a phthisical patient, whether or not the patient's illness has been previously notified.

The process of disinfection applied is that the internal surfaces of the patient's room and all articles in it are sprayed with formalin solution. When wall papers are dirty these are then stripped, the

ceiling is whitewashed, bedding, carpets, etc. are removed to the disinfecting station and subjected to saturated steam in an equifex
disinfector, and the entire room is scrubbed and washed[1].

(3) The patient is instructed as to the precautionary measures
required, printed cards being given in addition to exact verbal instructions. If poor he is supplied with a pocket spittoon for outdoor
use, and with Japanese paper handkerchiefs for indoor use.

(4) A careful sanitary inspection is made of each house in which
a phthisical patient lives, and sanitary defects are remedied. It has
been urged that apart from the notification of phthisis the Sanitary
Authorities already have power to inspect dwellings and enforce the
proper remedies against dirtiness and overcrowding ; and to inspect
workshops and factories and similarly provide remedies to prevent the
inhalation of irritating or morbific dust. Very few districts however
possess a sufficiently large staff of sanitary inspectors to secure even an
annual inspection of every dwelling and workshop ; and in the intervals
of the visits serious evils may have been long in existence. Insanitary
conditions are much more dangerous to the healthy when there is the
superadded risk of infection ; and the notification of cases of phthisis enables prompt and direct action for the removal of dirt and overcrowding
to be taken at the point where it is most urgently required. It is the
difference between drawing a bow at a venture and aiming straight at
the mark.

(5) If the patient is lodged under unfavourable conditions, and
especially if he is in danger of infecting others, he is, by arrangement
with his medical attendant, admitted for a month into the Borough
Sanatorium.

Indiscriminate Expectoration. There is no general enactment in
England against indiscriminate expectoration. By-laws, *i.e.* local
regulations, are however becoming adopted to an increasing extent
which prohibit spitting in or on tramcars belonging to local authorities. The Glamorganshire County Council was the first Sanitary
Authority to secure a more general by-law against spitting. Its
substance is as follows :

A person shall not spit on the floor, side, or wall of any public carriage or of any
public hall, public waiting room or place of public entertainment, whether admission thereto be obtained by payment or not.

Any person offending against this By-law shall be liable to a fine not exceeding £5.

[1] The methods of disinfection adopted in Manchester are fully described in *Trans.
British Congress on Tuberculosis*, Vol. II. p. 18.

Since then a few other authorities have followed this good example, the most recent being the London County Council and Brighton.

Disinfection and Cleanliness cannot be efficiently secured unless each case of phthisis is notified to the medical officer of health. In actual practice it is found that in many instances no precautionary instructions have been given to the patient, or such instructions as have been given are neglected unless reinforced at intervals. Even in notified cases there is considerable danger of neglect of the simple precautions required, until or unless the patient has had Sanatorium training. The amount and details of disinfection required will vary according to circumstances. The methods adopted in Brighton are given on page 455, and reference is made to the Manchester methods. To secure primary disinfection and subsequent cleanliness in every detail is the most important object of notification.

General Sanitary Improvement. Further details under this head are scarcely necessary. I have already (page 449) expressed my opinion that some of the most important reforms of the last half-century have lowered the mortality from phthisis in part by diminishing the opportunities for infection. At the same time, the enforcement of by-laws requiring a sufficient air space at the rear of as well as in front of dwellings, enabling every room in a house to be swept by air and purified by the sun, have doubtless greatly helped in the same direction. Even more important probably has been the influence of sanitary supervision in securing the cleansing of rooms and the abatement of overcrowding.

The Sanatorium Training of Consumptive Patients. Even when definite precautionary instructions have been given by myself to notified phthisical patients, it has occasionally been found on subsequent visits that these are not effectually carried out. It is one thing to make the patient understand the instructions given, another to ensure that he will conscientiously carry them out. To ensure this end the patient's self-interest, as well as his conscience, must be utilised. If he can be taught heartily to believe that his own welfare and that of his family is favoured by the precautionary measures recommended to him, we may usually rely on his co-operation. How to secure this educational influence became then an important question early in my local experience of the notification of phthisis in Brighton. Although a large amount of good was done by the visits to phthisical patients, there was reason to believe that some of them continued carelessly to disseminate infection in workshops, etc. by means of their sputa. After a few

months' experience of sending selected patients to an open-air sanatorium outside Brighton, I obtained, in July 1902, the consent of the Town Council to the admission of four consumptive patients into one of the isolation pavilions of our Borough Fever Hospital, which is very favourably situated for this purpose[1].

In my report on this subject I pointed out that the cases notified to us are usually suffering from the disease in a stage at which cure cannot be expected even by three months' treatment in an open-air Sanatorium; but that apart from the possibility of cure, it was in the public interest to admit phthisical patients not living under favourable conditions at home to the Borough Sanatorium for a month or two, according to circumstances. It would diminish disease and improve the public health in three ways:

(1) The patient himself would improve in health, and be enabled to start afresh, with an increased prospect of recovery.

(2) While he was in the Sanatorium his home could be cleansed and purified: his wife and family would have a holiday in the sense of being free from repeated attacks by the contagium of phthisis.

(3) The patient when sent home would have been taught to so manage his expectoration that he would no longer be a source of risk to his family and to those with whom he worked.

This course was at once adopted, and before the end of 1902 the number of beds utilised for this purpose had been increased to ten.

The majority of the patients are unable to come into the Sanatorium for longer than a month. They would lose their means of livelihood if they were absent from work for longer than that time. In a certain number of other cases, however, it has been possible to arrange for a longer treatment, and if the improvement made in the month has been such as to justify continuing the expense of the treatment, it has been continued for a second or even a third month.

Under present circumstances we are annually passing through the Sanatorium 100 to 120 phthisical patients. As the total deaths from phthisis in 1902 were only 174, as each consumptive patient lives several years, and as those of higher social status are not so likely to be the cause of infection to others, it can be confidently hoped that in a few years nearly every phthisical patient in Brighton who is a source of

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[1] No difficulty has arisen owing to patients being afraid of acquiring scarlet fever or diphtheria, and no cross-infection has occurred; each disease has a separate pavilion with its own separate recreation ground. The phthisical patients are treated on the same lines as in other open-air Sanatoria.

danger to others will have had a month's practical training in the simple precautions required to prevent him from becoming so.

From the above statement it will be gathered that the curative aspect of Sanatorium treatment is regarded as of secondary importance. We are chiefly concerned with educating these patients, and thus avoiding risk to others. At the same time the patients have a practical personal demonstration of the benefits to be derived from abundant food, an open air life, and freedom from infective dust. When they leave the Sanatorium at the end of the month, which in the majority of instances is the limit of time, they are without exception ardent advocates for the fresh air *régime*, and I have not yet known one out of the 71 patients thus treated (to June 25th, 1903) who, after leaving the Sanatorium, has again become careless as to coughing and expectoration; and this notwithstanding the fact that only a minority of the patients leave us without expectoration. They have so far improved in health that they are most eager to continue the *régime*, so far as their means will allow.

It appears to me that in connection with Sanatorium treatment, too much stress has been laid upon the cure of the patient. Such cure must be exceptional unless the treatment can be continued for six or more months, and unless it can be begun earlier in the disease than that at which cases of phthisis are usually notified. Each of our patients is informed before he is admitted to the Sanatorium (unless the disease is in its earlier stage) that a cure cannot be secured in the time during which he will be treated, but that he will be taught how to manage himself so that when he leaves he can, so far as his means admit, continue the treatment, and can pursue his daily life without risk to his relatives or fellow workmen. It is made a *sine quâ non* that as soon as the patient is admitted to the Sanatorium his house shall be thoroughly cleansed and disinfected. When he returns home, therefore, both he and his family are freed from the risk of infection by old infective material.

The educational part of Sanatorium treatment, as developed above, is more important in the public interest than its curative aspect. The majority of cases are notified in the second or even in the third stage of the disease. The natural history of phthisis is, as is well known, one of repeated exacerbations with intervals of quiescent disease. During the quiescent intervals the patient, if belonging to the artizan and labouring classes, resumes his work, and so is a continuing source of danger not only to his family but also to those employed with him. Hence it is of

the utmost importance that, although his illness is at an advanced stage, the patient should receive that practical instruction in the management of his expectoration, and should receive that practical demonstration of the benefits which will accrue to him personally which, in most instances, can only be secured by temporary residence in a well-managed Sanatorium. Our rule in selecting patients is to prefer (1) men to women, and (2) those still able to work; because in most instances, by treating and training these the circle of good achieved is wider than when we treat and teach those with a more limited environment. There are exceptions to this rule. Several instances could be quoted in which the mother of a large family suffering from chronic phthisis has infected every child in succession, while she has remained able to carry on her family duties. Hence a certain number of selected female patients are admitted when we have beds available for them[1].

Education of the public and of patients. The preceding review will have made it clear that the line in which most good is to be expected is in the writer's opinion by the training of patients, especially by their training in a Sanatorium. The education of the public is progressing apace, but in order to prevent exaggerated fears careful instruction is necessary. Panic is caused by imperfect knowledge, not by properly weighed instruction. Increased attention is being paid in our elementary schools to physical training and to the teaching of domestic hygiene, though they are still too much neglected; and if these two lines of teaching were to be adopted in every school, a much more rapid decline in the death-rate from phthisis could undoubtedly be secured.

In the preceding review of the struggle in England against tuberculosis, no attempt has been made to review what is being done to secure freedom of our meat and milk from tuberculosis. In regard to the condemnation of tuberculous meat, the rules laid down by the Second Royal Commission on Tuberculosis are generally followed. In regard to milk, several towns have special powers to secure the examination of milk from herds of cows suspected of being tuberculous, and to prevent the consumption of cow's milk from cows, the milk of which has been shown to contain tubercle bacilli. The details of the administration of these special powers are given by Dr Niven in vol. II. p. 282, of the "Transactions of the British Congress on Tuberculosis,"

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[1] For details as to the amount of provision of Sanatorium treatment in England see Postscript, p. 461.

1901. At the present time a Third Royal Commission on Tuberculosis is studying the intercommunicability of bovine and human tuberculosis.

Forecast as to future progress. The preceding review of the history of tuberculosis in England since 1838, and of the causes, including the administrative sanitary measures, which have caused it to decline in amount, will leave little doubt as to the trend of future measures having the same object in view.

Two ends are aimed at, and there need be no quibbling as to their relative importance. The first is to fortify each member of the community against the disease when he is invaded by its active cause, the tubercle bacillus. The second is to protect each member of the community against the invasion of the tubercle bacillus. I have already indicated my belief that many of the reforms which are supposed to have operated in the first direction, especially improvements in housing and in cleanliness, have also operated still more in the second direction. A difference of opinion as to the *modus operandi* need not prevent combined action in the struggle against tuberculosis in each direction. The encouragement of treatment in Sanatoria and still more of the training in Sanatoria on which I have laid stress is ideal in this respect. Such training teaches the patient the importance of the fortifying influence of an out-door life, of sunshine and of general hygiene, including a personal condition of high nutrition. It also teaches him the importance of the careful and cleanly control of his expectoration, in the interest of himself and of his friends. In the next twenty years we shall have not only steady improvement in general sanitation, but in all probability, the notification of every case of phthisis with efficient measures of disinfection and cleansing and such an extension of Sanatorium treatment as has hitherto appeared to be impracticable; and it is not difficult to foresee that along with the general adoption of these measures will come a reduction of tuberculosis at a rate much more rapid and more extensive than has hitherto been secured. There is, I believe, more hope of the almost complete extermination of tuberculosis than of any of the acute infectious diseases, with the possible exception of typhus and small-pox.

POSTSCRIPT.

Sanatorium Treatment of Phthisis in England.

In his address to the British Congress on Tuberculosis, Dr R. Koch stated "the only country that possesses a considerable number of special

hospitals for tubercular patients is England, and there can be no doubt that the diminution of tuberculosis in England, which is much greater than in any other country, is greatly due to this circumstance." This statement in its entirety is scarcely capable of substantiation. In its literal sense it cannot be held that the limited number of special hospitals for consumptives in England can have had so great an effect on the total death-rate from tubercular diseases as is here claimed. The proportion of such hospitals to the total number of tubercular patients is extremely minute. If however the total number of tubercular patients treated in general hospitals and still more those treated in workhouse infirmaries be included, there is good reason for attaching a high importance to the removal of these patients from their relatives and to the nursing of them under conditions in which personal infection is greatly limited in extent. A majority of the total tubercular patients belong to the poorer classes, and a very high proportion of these drift into the workhouse infirmaries. The following particulars as to the parish of Brighton illustrate this point. During the three years ending Oct. 1st, 1901, 211 phthisical patients were admitted to its workhouse infirmary. Each of these patients on an average spent 316 days in the infirmary. A large number (48) of them left the infirmary after an average stay of 136 days; 51 others left the infirmary after an average stay of 146 days and were subsequently readmitted, their average length of stay when they returned being 591 days. The average stay of those dying in the infirmary was 133 days before death, not including the time spent in the infirmary by these patients on previous occasions. During the three years in which the above 211 patients were admitted to the infirmary about 400 deaths were caused by phthisis in the parish of Brighton. On the assumption that each patient discharges infective material for three years before death, and that there were thus 1,200 phthisical patients in the parish of Brighton, it follows that nearly 18 per cent. of the total phthisical patients were removed from their homes during a large portion of the time when they were most infective. The patients thus removed were those whose home conditions were such as to render spread of infection almost inevitable. Conditions similar to those of Brighton hold good for other parts of the country.

The number of indoor paupers (including vagrants and insane) was 7·7 per 1,000 of the population of England and Wales in 1848 and 6·9 per 1,000 in 1902, the lowest in the interval being 5·8 in 1860. During the same period the number of outdoor paupers declined from 55·0 per

1,000 of population in 1849 to 17·7 in 1902. Hence a much higher proportion of the total number of pauper patients are now treated in workhouse infirmaries than formerly (Fig. 2). It has been estimated that one-eleventh of the total pauperism of this country is caused by
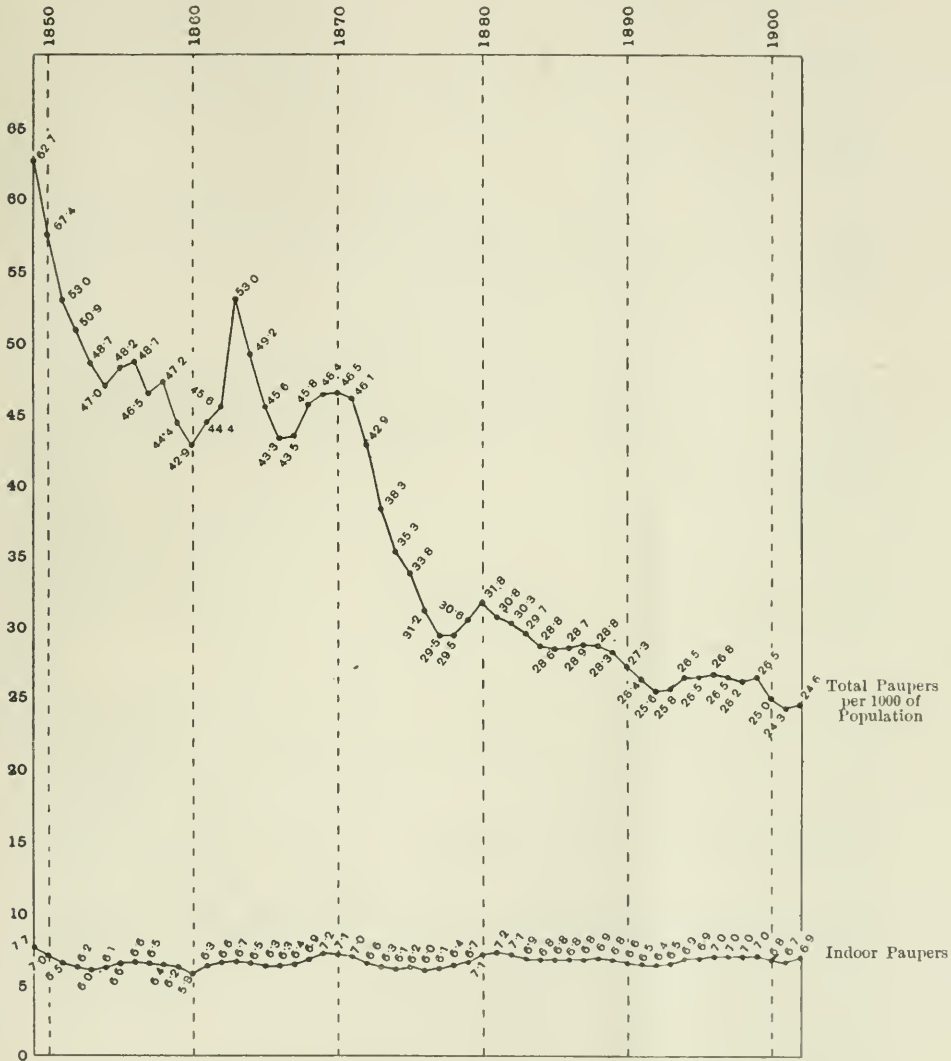
Fig. 2. Number of Paupers including Vagrants and Insane relieved in England and Wales each year from 1848-9 to 1901-2, classified as indoor and outdoor paupers per 1000 of Population.

phthisis (Dr Milson Rhodes). It is highly probable that the 5·8 to 7·7 per 1,000 of the total population who reside in workhouses comprises a very high proportion of consumptives. I am unable to obtain statistics as to the number of indoor paupers before 1838; they were almost certainly much fewer than in later years.

The withdrawal of this large number of patients in the later stages of phthisis from their homes must have had an important influence in diminishing the spread of the disease; and if among "special hospitals" be included workhouse infirmaries, Koch's statement quoted above is in a large measure justified.

In classifying institutions for the reception of tubercular patients we must therefore attach the first importance to the workhouse infirmaries. In a steadily increasing proportion of infirmaries these phthisical patients are treated in separate wards.

1. Poor-law provision. { A. For early cases.
                        { B. For advanced cases.

There is no poor-law provision for early cases as such, but an early phthisical patient if unable to work might be admitted to the workhouse infirmary. Workhouse infirmaries are invaluable as homes for advanced phthisical patients.

Recently efforts have been made for further specialization of the parochial treatment of phthisis. The three poor-law districts forming the city of Liverpool have combined to erect a large Sanatorium for pauper patients, and it is probable that other poor-law authorities will follow this example.

2. *Special Municipal Sanatoria for Phthisis* under the control of Sanitary Authorities and supported out of the general rates (as distinguished from the poor-rates) have not yet been built. Details have been given of the system under which 10 beds in connection with an already existing Fever Hospital are permanently reserved at Brighton for phthisical patients. Where separation of diseases can be secured this is probably a more economical plan, except for very large cities, than a totally separate institution. In Manchester (Dr Niven's Annual Report for 1901, page 230) during 1901, 20 cases of phthisis were treated in the otherwise empty local small-pox hospital, but the experiment had to be stopped owing to the admission of small-pox cases. The city of Sheffield decided in 1901 to establish a Sanatorium for phthisical patients, and finally obtained the consent of the Local Government Board to borrow money under Sec. 131 of the Public Health Act for this purpose. This scheme has not yet been carried out.

3. *Combined Public and Private Enterprise.* The most striking instance of this is the Westmoreland Sanatorium, an adapted building equipped by the kindness of Dr Paget Tomlinson, towards the support of which several Westmoreland Sanitary Authorities and Boards of Guardians contribute.

4. *Paying Sanatoria* have sprung up in various parts of the country and are doing good work. For particulars as to these reference may be made to the list published by the National Association for the Prevention of Tuberculosis and to Dr F. Rufenacht Walters' work on Sanatoria.

5. *General and Special Hospital Provision.* The various special hospitals for phthisis still carry on their good work. There is, on the other hand, an increasing objection on the part of the authorities of general hospitals supported by voluntary contributions to admit phthisical patients. Public and medical opinion, in fact, appears rightly to be undergoing the same change of opinion in favour of separate treatment as has occurred in regard to enteric fever[1].

6. *Homes for advanced cases of Phthisis* have already been mentioned, so far as parochial patients are concerned. For less poor patients homes are greatly needed for advanced cases where the home circumstances are unfavourable to the patients and their relatives.

APPENDIX.

SHEFFIELD CORPORATION ACT, 1903.
SECTION 45.

1. (A) Every registered medical practitioner attending on or called in to visit any person within the city shall forthwith on becoming aware that such person is suffering from tuberculosis of the lung send to the medical officer of health a certificate stating the name age sex and place of residence and employment or occupation (so far as can be reasonably ascertained) of the person so suffering and whether the case occurs in his private practice or in his practice as medical officer of any hospital public body friendly or other society or institution.

(B) Any such medical practitioner who fails to give such certificate shall be liable on summary conviction to a fine not exceeding forty shillings.

(C) The Corporation shall pay to every such medical practitioner for each certificate duly sent by him in accordance with this section a fee of two shillings and sixpence if the case occurs in his private practice and of one shilling if the case

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[1] The special hospitals in London provide accommodation for about 665 patients, while those in the provinces and Scotland have together only about 78 beds (Dr Kelynack, *The Hospital*, April 25, '03, p. 58).

occurs in his practice as medical officer of any hospital public body friendly or other society or institution.

(D)   A payment made to any medical practitioner in pursuance of this section shall not disqualify that practitioner from serving as a member of the Corporation or as a guardian of a union situate wholly or partly in the city or in any municipal or parochial office.

2.   (A)   Where the medical officer of health certifies that the cleansing and disinfecting of any building (including in that term any ship vessel boat tent shed or similar structure used for human habitation) would tend to prevent or check tuberculosis of the lung the town clerk shall give notice in writing to the owner or occupier of such building that the same or any part thereof will be cleansed and disinfected by the Corporation at the cost of the Corporation unless the owner or occupier of such building informs the Corporation within twenty-four hours from the receipt of the notice that he will cleanse and disinfect the building or part thereof to the satisfaction of the medical officer of health within the time to be fixed in the notice.   If within twenty-four hours from the receipt of such notice the owner or occupier of such building has not informed the Corporation as aforesaid or if having so informed the Corporation he fails to have the building or the part thereof disinfected as aforesaid within the time fixed by the notice the building or the part thereof shall be cleansed and disinfected by the officers and at the cost of the Corporation under the superintendence of the medical officer of health. Provided that any such building or part thereof may without any such notice being given as aforesaid but with the consent of the owner or occupier be cleansed and disinfected by the officers of and at the cost of the Corporation under the superintendence of the medical officer of health.

(B)   For the purpose of carrying into effect the provisions of this sub-section the Corporation may by any officer authorised in that behalf who shall produce his authority in writing enter on any premises between the hours of ten o'clock in the forenoon and six o'clock in the afternoon.

(C)   Every person who shall wilfully obstruct any duly authorised officer of the Corporation in carrying out the provisions of this sub-section shall be liable to a penalty not exceeding forty shillings and if the offence is a continuing one to a daily penalty not exceeding twenty shillings.

3.   (A)   The medical officer of health generally empowered by the Corporation in that behalf may by notice in writing require the owner of any household or other articles books things bedding or clothing which have been exposed to the infection of tuberculosis of the lung to cause the same to be delivered over to an officer of the Corporation for removal for the purpose of disinfection and any person who fails to comply with such requirement shall be liable on summary conviction to a penalty not exceeding five pounds.

(B)   Such articles books things bedding and clothing shall be disinfected by the Corporation and shall be brought back and delivered to the owner free of charge.

4.   If any person sustains any damage by reason of the exercise by the Corporation of any of the powers of sub-sections (2) and (3) of this section in relation to any

matter as to which he is not himself in default full compensation shall be made to such person by the Corporation and the amount of compensation shall be recoverable in and in the case of dispute may be settled by a Petty Sessional Court.

5.   No provisions contained in any general or local Act of Parliament relating to infectious disease shall apply to tuberculosis of the lung or proceedings thereto under this section.

6.   All expenses incurred by the Corporation in carrying into effect the provisions of this section shall be chargeable on the district fund and general district rate.

7.   The Corporation shall cause to be given public notice of the effect of the provisions of this section by advertisement in the local newspapers and by handbills and shall give formal notice thereof by registered post to every medical practitioner in the city and any other registered medical practitioner known to be in practice in the city and otherwise in such manner as the Corporation think sufficient and this section shall come into operation at such time not being less than one month after the first publication of such an advertisement as aforesaid as the Corporation may fix.

8.   The provisions of this section shall cease to be in force within the city at the expiration of seven years from the date of the passing of this Act of Parliament or by Provisional Order made by the Local Government Board and confirmed by Parliament, which Order the Local Government Board are hereby empowered to make in accordance with the provisions of the Public Health Act, 1875.

9.   The term "Medical Officer of Health" in this section shall mean the Medical Officer of Health for the time being of the city or any person duly authorised to act temporarily as Medical Officer of Health for the city.

BIRTH-RATE AND DEATH-RATE IN NEW ZEALAND.

By W. J. BARCLAY, M.D., F.R.C.S.E.

(Abstracted from a thesis presented for the degree of Doctor of Medicine, University of Edinburgh.)

Of all British colonies New Zealand is the one that most closely resembles Great Britain in size, in situation, and in climate. And the inhabitants of the two countries are of practically the same race. The vital statistics of New Zealand are therefore eminently suitable for comparison with those of Great Britain. In the present paper comparison has been restricted to the birth-rates and death-rates, and in this limited survey several points of interest present themselves.

Before proceeding farther, it is well to consider the trustworthiness of the data, in other words the accuracy of the New Zealand birth-rates and death-rates. The New Zealand rates mentioned in this paper are for the most part those officially published by the Registrar-General for the colony, and they may be assumed to represent the truth, provided that the fundamental data are correct. For the accuracy of birth-rates and death-rates depends on two factors, viz. the completeness of the registration of births and deaths, and the correctness of the calculation of the population.

1. *The completeness of the registration of births and of deaths* cannot be tested, but there is reason to suppose that registration is fairly complete in New Zealand, where for many years all statistical returns have been carefully and fully recorded.

2. *The calculation of the population.* There is in New Zealand a five-yearly census, as contrasted with the ten-yearly census which is

the rule in most other countries. There must be some unavoidable error in estimating by any method the population of a country during inter-censal periods, but frequent census enumerations reduce this error to a minimum. Further, in New Zealand accurate returns of immigration and emigration are available, and the population for inter-censal years is calculated by adding to the number enumerated at the last census the excess of immigration over emigration, and the excess of births over deaths. This method has been found to give very accurate results when checked by the five-yearly census.

A few more facts regarding the population of New Zealand are worthy of consideration.

1. *Number of the population.* Though New Zealand has been inhabited by English-speaking people for only a little more than sixty years, the present population is large enough to provide birth-rates and death-rates free from those temporary variations which are found in dealing with small populations. Thus whilst the first census of New Zealand, taken November 1st, 1851, showed that the population was only 26,707, the last census of March 31st, 1901, gave a total of 772,719. It is to be noted that the above figures, as well as the birth-rates and death-rates mentioned later, refer only to the " European" population of New Zealand : this excludes the natives or Maoris, but includes a few Chinese and other Asiatics as well as all of European birth.

2. *Nationality.* As regards nationality, determined by birthplace, the census of 1901 showed that of the whole European population of New Zealand 97·43 % were of British birth, 67 % being New Zealand born, and 26·56 %[1] born in the United Kingdom : only 2·41 % were of foreign birth.

3. *Age and Sex Distribution of the Population.* This is important on account of the effect it has on the birth- and death-rates. In brief, there is in New Zealand as compared with England and Wales,

(*a*) A deficiency of people at higher ages.

(*b*) A deficiency of females.

Both these peculiarities are due to immigration and other conditions of colonial life, and they are becoming less marked as the conditions of life in New Zealand are approximating more nearly to those obtaining in Great Britain. Thus the proportion of old people in the New Zealand population is increasing, as shown in the following table :—

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[1] One of these percentages is incorrect, but a rectification is impossible because of the author's absence from England.—ED.

TABLE 1.

New Zealand	
Year	Persons 65 years and upwards per cent. of population
1864	0·63
1871	1·08
1881	1·41
1891	2·29
1896	2·95
1901	4·06

As regards sex distribution, the increasing proportion of females is shown by the following table :—

TABLE 2.

New Zealand	
Year	Number of females to 100 males
1861	62·16
1871	70·52
1881	81·72
1891	88·26
1896	89·31
1901	90·33

The effect of these changes in the age and sex distribution of the New Zealand population will be referred to later.

In the meantime we may conclude that the New Zealand birth- and death-rates are trustworthy, being calculated from data which are fairly accurate.

BIRTH-RATES.

I. *Crude Birth-rates.*

1. *General Birth-rate* or number of births per 1000 of the population: It is obvious that this rate will be affected by the number of women, and especially married women, of child-bearing ages, in proportion to the rest of the population. Thus New Zealand,

whose population when compared with that of England and Wales shows a relative deficiency of females, will for this reason alone have a lower general birth-rate—other conditions being equal. This error may be eliminated by calculating corrected birth-rates, where the number of births is expressed proportionately to 1000 women of child-bearing age. The relative values of crude and of corrected birth-rates will be discussed later, but it may safely be asserted that the general birth-rate, though a crude, uncorrected rate, is for many purposes of extreme importance.

The following table gives the general birth-rate in New Zealand and in certain other countries:—

TABLE 3.

Birth-rate per 1000 of Population

Year	New Zealand	Victoria	England and Wales	Ireland	France
1880	40·78	—	34·2	24·7	24·6
1885	34·35	—	32·9	23·5	24·3
1890	29·44	33·60	30·2	22·3	21·8
1895	26·78	28·57	30·2	23·2	21·7
1899	25·12	26·71	29·1	22·9	21·9
1900	25·60	25·82	28·7	22·7	21·4
1901	26·34	25·77	28·5	22·7	22·0

This table will suffice to show that the New Zealand birth-rate has fallen very rapidly during the last twenty years, till in the year 1900 it was lower than the birth-rate of any other Australasian colony, and lower than that of any European country save Ireland and France, two countries which are notorious for their low birth-rate.

2. *Illegitimate Births:* If we adopt the usual though unsatisfactory method of stating illegitimate births in proportion to total births, it will be found that whilst the general birth-rate is falling the proportion of illegitimate births is rising in New Zealand, as in other countries.

TABLE 4.

Proportion of Illegitimate Births in every Hundred Births

Year	New Zealand	Queensland	New S. Wales	Victoria	S. Australia	W. Australia	Tasmania
1888	3·05	4·13	5·08	4·80	2·67	—	3·62
1890	3·30	4·85	5·26	5·09	2·50	—	4·05
1895	4·50	4·93	6·51	5·33	3·13	4·47	4·97
1900	4·63	6·40	7·01	5·91	4·24	4·82	5·43
1901	4·57	5·93	7·16	5·58	—	—	—

It may easily be shown, however, that this is but an apparent increase of illegitimacy. Thus in New Zealand in the year 1886 there were 602 illegitimate births, in 1896 there were 834 illegitimate births, an increase of 38·5 %. But during the same decennium the number of unmarried women aged 15 to 45 years increased from 52,348 to 85,105, or at the rate of 62·6 %. There was therefore in reality a considerable reduction in the amount of illegitimacy: the same conclusion will be derived from a study of the corrected illegitimate birth-rate (see later, Table 7).

But, making use of the available returns, it may be noted that the proportion of illegitimate to total births is not high in New Zealand as compared with many other countries. The following table gives the average results for a period of five years:—

<div align="center">TABLE 5.</div>

Country	Percentage of Illegitimate to Total Births
New Zealand	4·42
New South Wales	6·88
Victoria	5·55
Queensland	5·94
South Australia	3·76
West Australia	5·06
Tasmania	5·65
England and Wales	4·15
Ireland	2·65
Scotland	6·97
Germany	9·21
Austria	14·55
France	8·26

II. *Corrected Birth-rates.*

A more accurate method of expressing the birth-rate is to give,

1. The proportion of legitimate births per 1000 married women, aged 15—45 years: the legitimate birth-rate.
2. The proportion of illegitimate births per 1000 unmarried women, aged 15—45 years: the illegitimate birth-rate.

1. *The Legitimate Birth-rate:* This is the more important of these two corrected rates, and it may be used as a fair mode of comparing the birth-rates of two different countries since it avoids fallacies due to differences in the age and sex distribution of the populations. The following table gives the legitimate birth-rate for New Zealand and for England and Wales in certain years:—

TABLE 6.

Legitimate Birth-rate per 1000 Married Women 15—45		
Year	New Zealand	England and Wales
1878	337·2	—
1881	313·3	285·6
1886	295·5	—
1891	276·3	268·0
1896	252·1	—
1901	243·8	—

This table shows that the birth-rate is falling rapidly in New Zealand, in confirmation of the evidence of the crude general birth-rate (see Table 3). But also it may be shown that there is a difference in the results given by the crude general birth-rate and the corrected legitimate rate. Taking the year 1891 as an example, reference to Table 6 demonstrates that in this year the corrected legitimate birth-rate was *higher* in New Zealand than in England and Wales (276·3 as contrasted with 268·0): but in the same year the crude general birth-rate was *lower* in New Zealand than in England (29·01 as contrasted with 31·4).

Both rates are of value, but for different purposes. The corrected legitimate birth-rate is rightly employed when it is desired to compare the fecundity of the child-bearing portion of the population in two countries, the crude general birth-rate on the other hand must be used if we wish to compare the fecundity of the whole populations. Thus in the year 1891 the married women of child-bearing age showed a greater fecundity in New Zealand than in England; yet, owing probably for the most part to the relative deficiency of females in the colony, the general birth-rate, the measure of national fecundity, was lower in New Zealand than in England. It is almost certain that what was true for 1891 held good also for earlier and later years. Hence in noticing that the general birth-rate has for some years been lower in New Zealand than in England, we must remember that the relative deficiency of females in the colony has contributed to cause the low birth-rate. This factor is, however, of diminishing importance: for we have already seen (Table 2) that in New Zealand the proportion of females to males is steadily increasing.

2. *Illegitimate Birth-rate,* or number of illegitimate births per 1000 unmarried women, aged 15—45 years.

The following table gives the rates for New Zealand and for England and Wales in certain years :—

TABLE 7.

Illegitimate Birth-rate per 1000 Unmarried Women aged 15—45		
Year	New Zealand	England and Wales
1881	—	14·1
1886	11·50	—
1891	—	10·6
1896	9·79	—
1901	9·01	—

This table (1) shows that the illegitimate birth-rate is about the same in New Zealand as in England and Wales, confirming the crude returns of Table 5.

(2) disproves the apparent increase of illegitimacy as shown in the crude figures of Table 4.   In fact the illegitimate birth-rate in New Zealand is falling even more rapidly than the legitimate rate, as appears from the following table :—

TABLE 8.

New Zealand				
Year	Illegitimate Birth-rate	Rate of fall	Legitimate Birth-rate	Rate of fall
1886	11·50	14·7 %*	295·5	14·6 %†
1896	9·79		252·1	
1901	9·01	8·0 ,,	243·8	3·2 ,,

* or 7·35 % for 5 years.   † or 7·3 % for 5 years.

SUMMARY.

We conclude therefore that in New Zealand,

(1) The crude general birth-rate is very low, and is still falling.

(2) The corrected legitimate birth-rate, though not quite so low (*e.g.* when compared with the corresponding rate in England and Wales), is nevertheless still falling.

(3) The corrected illegitimate birth-rate is falling even more rapidly.

III. *Causes of the Decline of the Birth-rate in New Zealand.*

It is not proposed to discuss these at any length, as the causes acting in New Zealand are apparently the same as those which have produced a fall in the birth-rate of all civilised countries. In brief, these causes are economic and social. It seems an almost universal rule that when people grow wealthy the birth-rate falls. Thus in any individual country the birth-rate is lower in the richer classes than it is in the poor or working classes; and the same is true in the history of nations, when the country grows wealthy the birth-rate falls. Wealth and prosperity lower the birth-rate in several ways: by causing people to delay marriage till later in life—or to avoid it altogether, and especially by leading to a voluntary avoidance of child-bearing on the part of married people.

In New Zealand, though there are none of inordinate wealth, there are on the other hand none or practically none in abject poverty, and the average man is distinctly well off as regards the necessaries or even the luxuries of life. The average of comfort and wealth has increased, till now it is perhaps higher than in most other countries. Probably it is to this increase in wealth that we must ascribe the fall in the New Zealand birth-rate.

IV. *Effect of the Decline of the Birth-rate.*

The population of a country is increased either by immigration or by the natural increase of excess of births over deaths. In New Zealand during the period 1885—1901 (inclusive), the population increased by 223,953, of which 208,213 or 92·9 % was from excess of births over deaths, whilst only 15,740 or 7·02 % was from excess of immigration over emigration. It is clear then that the important factor determining the increase of population in New Zealand is the natural increase by excess of births over deaths, and consequently the decline that has taken place in the birth-rate has seriously interfered with the increase of population. From a national point of view this is a calamity, and especially in a young colony where an increase of population is greatly desired.

It will be pointed out in the next section that the New Zealand death-rate is extremely low, being 9·8 for the ten years 1891—1900.

This low death-rate has to some extent counteracted the effect of the falling birth-rate; but we shall see that in all probability the death-rate will rise within the next few years, and if the birth-rate continues to fall, then the rate of natural increase in New Zealand will be seriously diminished.

The gravity of this contingency will be better appreciated by means of the following comparison. France has for some years been notorious as the nation which on account of a low birth-rate has failed to increase her population at the same rate as the other great Powers of Europe. The rate of natural increase in France has gradually diminished till it has become a negative quantity. This has naturally excited considerable alarm, and attempts are being made to improve the situation:

DIAGRAM I.

France (after Dr J. Bertillon).   Birth-rates ------.   Death-rates ———.
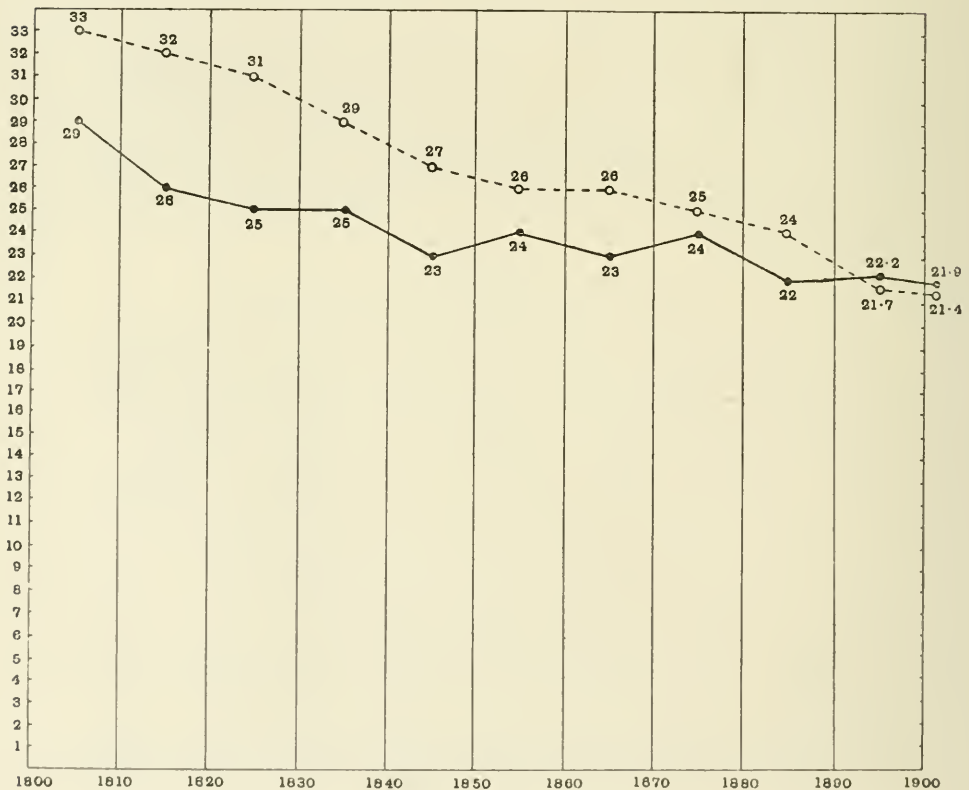
DIAGRAM II.

New Zealand.   Birth-rates, above.   Death-rates, below.

inter alia, a Society (L'Alliance nationale pour l'accroissement de la population française) has been formed to promote an increase of the population. The president of this Society, Dr J. Bertillon [1], asserts that France is being depopulated by the lowness of the birth-rate, to the detriment of the nation in matters military, commercial and intellectual. He maintains that "population is the source of all wealth, because all wealth originates in work which is produced by the hands or brains of men. In order that a country may be prosperous in every sense of the word, that it may be rich, powerful and intelligent, it must have a numerous population." Dr Bertillon also suggests a remedy for this depopulation, viz. that a tax be imposed on bachelors over thirty years of age and also on married people who have less than three children, on the other hand those having more than three children are to be exempted from taxation.

The preceding diagrams show graphically the relative condition of birth-rate and death-rate in France and in New Zealand.

In each of these diagrams the upper line indicates birth-rate, the lower line death-rate; and the intervening space represents the rate of natural increase. It will be noticed that in France the birth-rate has actually fallen below the death-rate. In New Zealand it is true there is still a large surplus of natural increase, but this is due solely to the remarkably low death-rate, which probably cannot long remain at its present low level. Even under present conditions it would need only two or three decades for the curves of birth-rate and death-rate to meet, and this calamity would be hastened were the death-rate to rise.

We conclude, therefore, that as regards increase of population in New Zealand, the outlook for the future is not satisfactory, and that it is the duty of the vital statistician to give timely warning by pointing out the facts and the consequences that must ensue.

Death-Rates.

I.   *Crude Death-rates.*

By this is understood the number of deaths annually per 1000 of population. The crude death-rate for New Zealand is remarkably low, and, as in most other countries, it is still falling.

TABLE 9.

New Zealand	
Year	Crude Death-rate
1855	12·63
1865	14·46
1875	15·19
1885	10·57
1·95	9·91
1900	9·43
1901	9·81

For purposes of comparison the mean crude death-rate for the ten years 1891-1900 has been calculated for New Zealand and for certain other countries, as given in the following table:—

TABLE 10.

Country	Crude Death-rate 1891—1900
New Zealand	9·8
Queensland	12·2
New South Wales	12·2
Victoria	13·9
South Australia	11·9
West Australia	15·7
Tasmania	12·5
England and Wales	18·1
Scotland	18·4
Ireland	18·2
France	21·5
Germany	22·2

These crude death-rates are not, however, free from fallacy. For it is well known that the death-rate varies greatly with the age and sex distribution of the population. Young children and old people have higher death-rates than adults of middle age, and males have at almost all ages higher death-rates than females. Consequently the crude death-rate is not a fair index of the mortality of a country during a period of years, unless it can be shown that the age and sex distribution of the population has remained unchanged. And if we wish to compare the crude death-rate of one country with that of another, the

population of the two countries should be the same as regards age and sex composition.

It has already been pointed out that the New Zealand population is altering in composition both as regards sex and as regards age. There has been an increase in the proportion of old people, but a decrease in the number of young children as well as an increase in the number of females. These alterations in the composition of the population are due partly to the diminishing effect of immigration, partly to the falling birth-rate, and partly to the natural increase in age of a population which formerly contained very few old people. The effect of these changes has probably been to slightly lower the death-rate : the increase in the proportion of females, and the decrease of young children, would both produce this effect, which probably is not yet counterbalanced by the increased proportion of old people. It is probable, however, that the changes taking place in the age distribution of the New Zealand population will in time produce a higher crude death-rate, in spite of the increasing proportion of females. For whilst there is naturally an increasing number of old people, there must also soon be a diminished proportion of young adults if the birth-rate continues to fall.

The effect of birth-rate on death-rate has been greatly disputed, but a full consideration of the subject will show that,

(1) A fall in the birth-rate will for a time reduce the death-rate but will ultimately increase it.

(2) A rise in the birth-rate will for a time increase the death-rate but will ultimately reduce it.

Probably the New Zealand death-rate has been and still is lowered by the change that has taken place in the birth-rate, but if the present condition of the birth-rate is maintained the opposite effect will be felt and the death-rate will rise.

As regards comparison of the crude birth-rate of New Zealand with that of England and Wales. The New Zealand population differs considerably in age and sex distribution from that of England. Thus taking the population in the census year 1896, we find that the New Zealand population, when compared with that of England and Wales according to the standard million 1881–90, showed

(1) Less numbers of both sexes at the extremes of life, ages 0–5 and 65+.

(2) Less numbers of females at all ages over 25 years.

The total effect of these differences was to lower the New Zealand death-rate ; the exact amount of lowering is shown later.

II. *Corrected Death-rate.*

It is evident from the above that the crude death-rate does not provide a strictly fair method of indicating the changes in the rate of mortality of a country, or of comparing the rates of mortality in two different countries. Correction for age and sex distribution of the population is necessary. This may be accomplished either by exhibiting in tabular form the death-rates for each sex at age-periods of five or ten years, or by calculating a single corrected death-rate for the whole population.

I.   *Death-Rates at Different Age-Periods.*

TABLE 11.

Age	Death-rate per 1000 living at each age-period					
	Males					
	New Zealand			England and Wales		
	1874, —78—81	1880—92	1891—96	1871—80	1881—90	1891—1900
0—5	33·56	29·09	28·83	68·14	61·6	60·8
5—	4·11	3·37	2·84	6·67	5·4	4·2
10—	2·61	2·21	2·17	3·69	3·0	2·4
15—	3·63	3·80	3·43	5·23	4·3	3·8
20—	4·96	5·39	4·89	7·32	5·7	5·2
25—	5·85	5·83	5·12	9·30	7·8	7·0
35—	9·58	8·31	7·40	13·74	12·4	11·9
45—	15·53	13·44	12·57	20·05	19·4	19·4
55—	26·08	25·33	24·65	34·76	34·7	35·8
65—	47·83	52·16	51·41	60·57	70.4	69·0
75+	107·45	—	130·80	169·08	162·2	?
	Females					
0—5	29·26	24·75	23·55	58·10	52·0	50·9
5—	3·67	2·85	2·76	6·20	5·3	4·2
10—	2·54	2·18	1·91	3·70	3·1	2·5
15—	3·43	3·62	3·46	5·43	4·4	3·7
20—	4·75	4·87	4·66	6·78	5·5	4·6
25—	6·75	6·12	5·40	8·58	7·4	6·3
35—	8·62	8·15	7·18	11·58	10·6	9·9
45—	12·06	10·84	10·13	15·59	15·1	14·9
55—	17·90	19·13	19·04	28·54	28·5	29·0
65—	42·58	42·80	43·52	60·82	60·1	60·0
75+	117·8	—	114·05	155·83	147·3	?
Columns	1	2	3	4	5	6

Column 1.   From data of Newman and Frankland[2].

„   2.   Leslie[3].

„   3.   W. J. Barclay.

„   4, 5, 6.   Registrar-General for England and Wales.

The results shown in this table are free from fallacy of age and sex distribution, and from them we conclude,

a. The death-rates are falling in New Zealand as in England and Wales, for both sexes and at practically every age-period.

b. The death-rates are considerably lower in New Zealand than in England and Wales.

2. *Single Corrected Death-rate.*

This expresses the same results in a more concise form. I have calculated a corrected death-rate for New Zealand during the year 1896, a census year. The sum total of the population was distributed amongst the various age-groups of the two sexes, not in the proportions which actually obtained in New Zealand, but according to the proportions which existed in the population of England and Wales during the period 1881–90 as given in the standard million of the Registrar-General. Knowing the actual death-rates recorded for each age-group in New Zealand during the year 1896, we can calculate the number of deaths that would have occurred at each age-group had the age and sex distribution of the New Zealand population coincided with that of England and Wales; and by addition we obtain the total number of deaths that would have occurred in New Zealand under the same conditions.

In this manner was obtained for New Zealand during the year 1896 a corrected death-rate of 10·382 per 1000, as contrasted with the crude death-rate of 9·10. Hence it is evident that the composition of the colonial population is such as of itself to give a death-rate which is too low when compared with that of England and Wales.

Yet even when correction is made for this factor, the New Zealand death-rate remains extremely low, viz. 10·382 for 1896. Compare also the rates in Table 11.

Cause of the Low Death-rate in New Zealand.

Two factors are concerned, viz. the people, and their environment.

1. *The People.* Are the inhabitants of New Zealand in themselves particularly healthy? Probably they are. For New Zealand was originally populated by a picked class of men and women: none but the strong and energetic would be likely to face the trials and hardships of early colonial life, especially in a colony so far distant from the mother

country. Some of these original colonists still survive, and in any case their immediate descendants may be presumed to have inherited vitality above the average seen in older countries. On the other hand an element of an opposite nature has been at work, especially of late years. The reputation of the New Zealand climate has attracted a considerable number of invalids, particularly those suffering from phthisis. Some of these, a minority, do not long survive, and their deaths help to unduly increase the death-rate of the colony. And, what especially concerns us here, the majority who do survive cannot be considered a healthy class. Probably, however, the original healthy colonists still have a pre-ponderating influence.

But it is evident that this cannot be the sole cause of the low death-rate in New Zealand, since other colonies have had equally favourable beginnings. The country itself is partly responsible, the above-mentioned second factor of environment.

2. *Environment.* This is to be considered in the widest possible sense, including Climate, Social and Industrial Conditions of Life. In a paper published in 1883, Newman and Frankland [2] maintained that in New Zealand there were the following causes for the low death-rate:

1. The struggle for existence is easier: good food and clothing are readily obtained.

2. A large proportion of the population are engaged in agriculture and other out-door occupations.

3. The population is sparse, no overcrowding of towns.

4. There are fewer manufactures and mines, hence less industrial disease.

5. The climate is temperate, and the soil unpolluted.

These causes may perhaps be divided into two main groups, (*a*) Climate, and (*b*) Industrial and Social Conditions.

(*a*) *Climate.* The climate of New Zealand, though exhibiting considerable variations according to locality, is on the average extremely temperate and equable. There are no great extremes of heat or cold, and prolonged droughts are unknown. There are no new diseases to be acquired by immigrants.

(*b*) *Industrial and Social Conditions.* The census returns show that in New Zealand a large proportion of the population is engaged in agricultural and pastoral work. These are undoubtedly healthy occupations, and especially in a climate so equable as that of New Zealand.

There can be no doubt that the average inhabitant of New Zealand has better food, house accommodation and clothing than his fellows in older countries. It is true that there is in the colony a certain amount of poverty, but this is assuredly much less than, *e.g.* in England and Wales, where no less than 20 % of the population is estimated to be living in a state of poverty.

We conclude that the following causes may be assigned for the low death-rate of New Zealand :—

1. Natural healthiness of the people.
2. Climate.
3. Favourable industrial and social conditions.

Life Table.

The preceding results based on the death-rates of New Zealand are confirmed by a Life Table for that country which the writer has calculated, based on the statistics for 1891–95. This life-table is published in *Public Health* (Oct. 1903), and it is only necessary to give here a few of the salient points. The male expectation of life at birth in New Zealand was 55·21 years as compared with 44·17 years in England and Wales (1891–1900), and with 40·98 years in London (1891–1900).

In females the expectation of life at birth in New Zealand was 57·79 years as compared with 47·82 in England and Wales, and 45·33 years in London. The expectations of life at higher ages, which are consistently greater in New Zealand than in England and Wales or in London, are given in the paper above referred to.

Although this factor does not cover the entire ground, there is every reason for ascribing the superiority of the New Zealand statistics mainly to its lower infantile mortality. The following comparative table of rates of infantile mortality brings out this point.

TABLE 12.

Rate of Infantile Mortality, or Deaths under 1 yr. per 1000 Births		
Year	New Zealand	England and Wales
1896	77·315	148
1897	72·263	156
1898	79·662	160
1899	95·885	163
1900	75·156	154
1901	71·397	166

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(2) NEWMAN and FRANKLAND. "An Enquiry into the Healthiness of New Zealand," by Alfred Newman, M.B., M.R.C.P., and F. W. Frankland. *Journal of the Institute of Actuaries*, October, 1883.

(3) LESLIE. "Rates of Mortality in New Zealand," by Geo. Leslie, Assistant Actuary, New Zealand Government Life Insurance Department. Published in the *New Zealand Journal of Insurance, Mining, and Finance*, Sept. and Nov. 1895. [This includes a life-table for New Zealand, 188–190.]

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CHEMICAL ANALYSES OF THE AIR IN THE HOUSE OF COMMONS.

By W. J. ATKINSON BUTTERFIELD, M.A., F.I.C.

THE Select Committee of the House of Commons which was first appointed on April 16th, 1902, and subsequently re-appointed with certain changes in its constitution, for the purpose of inquiring into the Ventilation of the House, instructed the author to make for their information a number of analyses of the air in the Debating Chamber and many other parts of the House. In this communication only the methods used and the results obtained in the case of the Debating Chamber will be referred to. The Committee reported to the House on the 28th July last, and its report, which is published by H.M.'s Stationery Office (No. 283), should be consulted for particulars of its conclusions and recommendations in regard to the ventilation of the House.

The object of the analyses made by the author was (to quote the words of the Committee) "to determine as exactly as present scientific methods will allow the actual condition of the air in the Chamber when in use." Before proceeding to describe the methods used, it will be convenient to give a short account of the way in which fresh air is supplied to and vitiated air withdrawn from the Chamber.

The air-supply is drawn from the outer air at a point on the ground level of the Houses of Parliament on the Terrace facing the river. A passage, having its mouth here, serves to pass the air from this intake to the House. It takes a somewhat circuitous course in the basement of the building, and in it are set up various arrangements for the treatment of the air, some of which will be somewhat altered in consequence of the recommendations of the Committee. The description here given applies to the arrangements existing at the time (1902) the author's observations were made (and for many years previously).

The treatment of the air is varied in some respects according to the prevailing atmospheric conditions. In warm dry weather cold water is sprayed into the passage a short distance from the intake. If the weather is hot, the air passes over blocks of ice placed in the passage way. In cold weather, the spray of water and the use of ice are discontinued. Under all circumstances the air is next filtered through an open-meshed canvas (scrim-cloth) screen, which is stretched diagonally across a widened space in the air way, so that the air traverses the screen at relatively low velocity. This filtration of the air serves to remove from it the grosser particles of soot and dust, and under ordinary atmospheric conditions no further filtration is required. But when fog prevails, it is necessary to employ additional means for the removal of the finer particles of smoke which abound in the air. The air is then forced through a layer of cotton-wool about six inches thick spread between two frames of wire netting, built into a long wooden framework which is V-shaped in cross section. It presents a filtering surface of about 1000 square feet, and has been ascertained by Dr Haldane to pass 1,500,000 cubic feet of air per hour, when the difference of pressure between the two sides is 4·2 mm. (0·17 inch) head of water, or 1 lb. per square foot. This is at the rate of 1500 cubic feet of air per hour per square foot of filter area. The flow of air varies directly as the pressure[1]. This cotton-wool filter is only in use in foggy weather and appears, from the evidence of Members of the House to be highly effective; at other times doors are left open which allow the air to pass on without traversing the layer of cotton-wool. The filter is apt to become more or less blocked in continuous use in damp weather, owing to the condensation of water in its pores.

The flow of air from the intake below the Terrace to and past or through the cotton-wool filter is maintained by a large propeller fan placed in the air passage. The speed at which this fan is run has to be considerably increased when the cotton-wool filter is brought into use in order to maintain the difference of pressure between the two sides of the layer of cotton-wool which is necessary to secure the passage through it of the normal quantity of air supplied per hour to the Chamber. The temperature of the inflowing air is kept very nearly constant, at about 62° Fahr., under all conditions.

The canvas screen and the end of the cotton-wool filter are shown in the annexed figure (Fig. 1), which is a cross-sectional view of the

[1] *Report of the Departmental Committee on the Ventilation of Factories and Workshops,* 1902 [Cd. 1302], p. 102.

lower part of the Debating Chamber of the House, and of the under-lying chambers. Above the filter is a battery of steam pipes and radiating plates by means of which the air is warmed in cold weather.
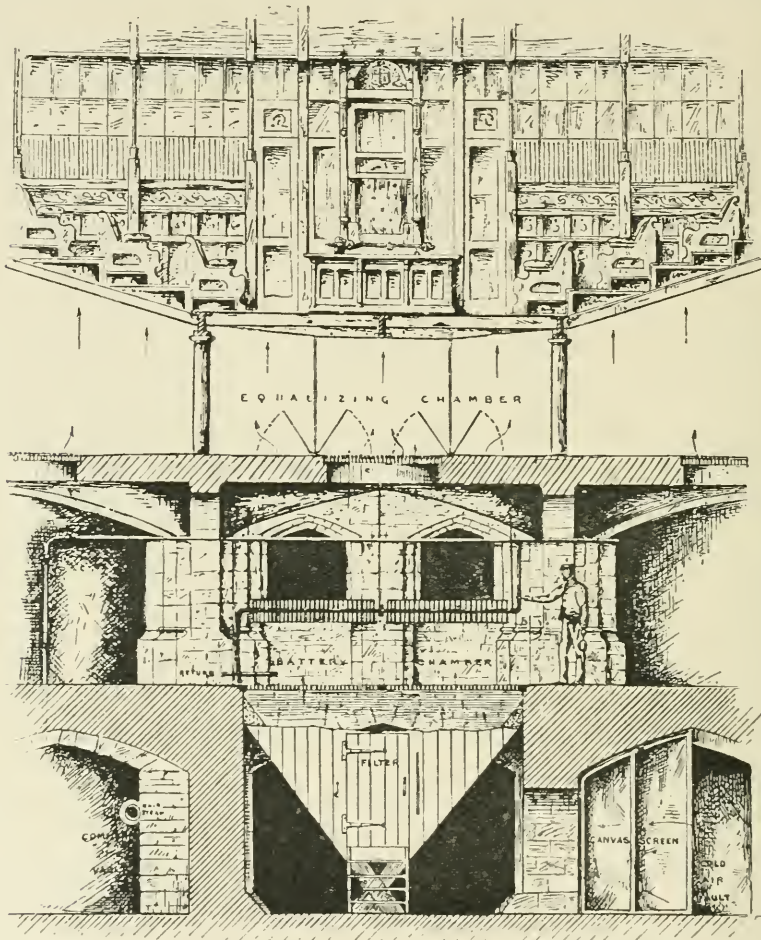
Fig. 1.

It then passes upwards to the chamber immediately below the Debating Chamber, known as the equalizing chamber. From the latter the air passes up through gratings in the floor, and beneath the seats, of the Debating Chamber. The gratings are covered by open-mesh matting, and one of the main objections raised against the present system of ventilation was that the inflowing air to the Chamber is

liable to be vitiated by thus passing through the matting which covers the floor over which Members are constantly passing.

Reference to the longitudinal and transverse sections of the Debating Chamber of the House of Commons, given herewith (Figs. 2 and 3), by
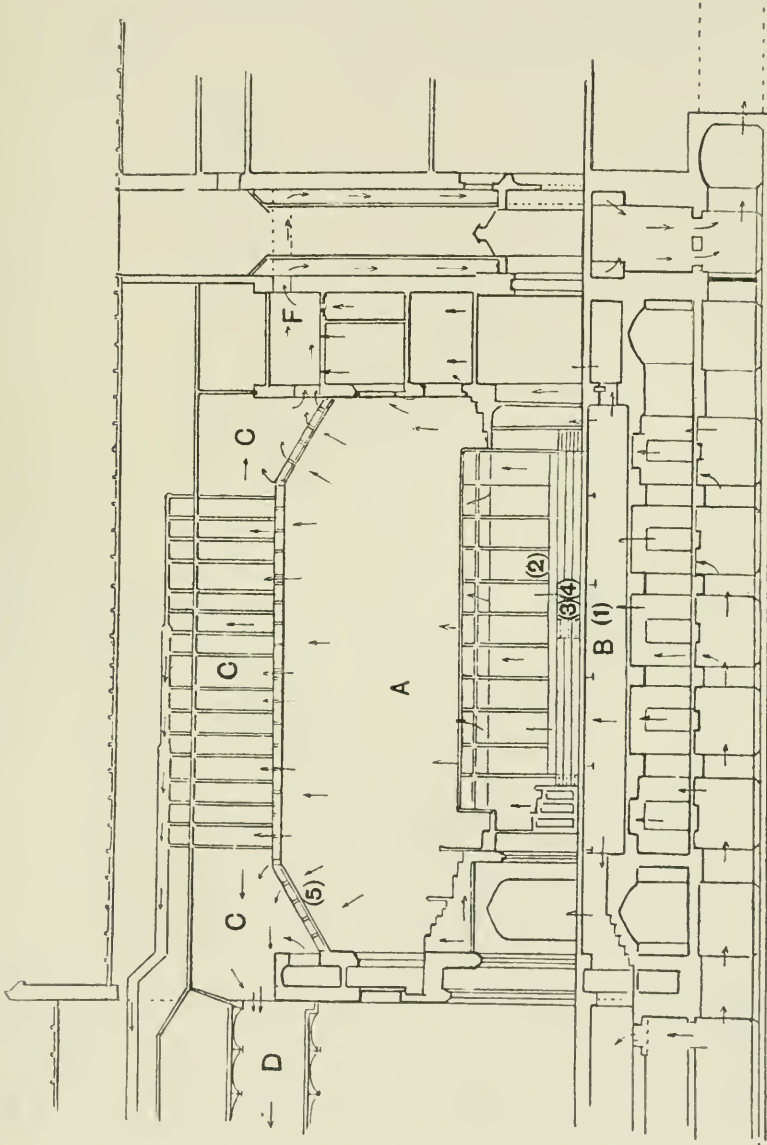
Fig. 2.

See Description below Fig. 3, next page.

kind permission of the First Commissioner of H.M.'s Office of Works, will show the course taken by the air into and through the Chamber. The arrows indicate the manner in which the air supply enters, and is distributed over the Chamber, and the vitiated air withdrawn from it. The outflow of vitiated air takes place through the ceiling of the Chamber, which is lighted by means of a large number of Argand gas
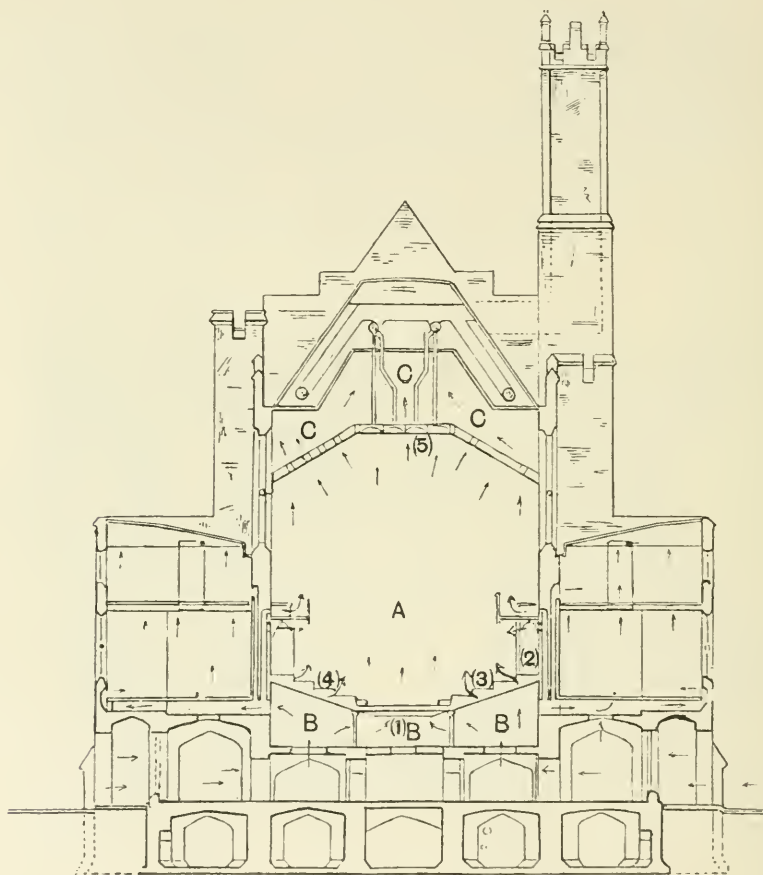
Fig. 3.

(A) Debating Chamber.   (B) Equalizing Chamber.   (C) Space above ceiling of Debating Chamber, with flues to gas burners.   (D, Fig. 2) Main exit for vitiated air. (F, Fig. 2) Secondary exit for vitiated air, leading to downcast shafts leading to the Clock Tower.

Approximate positions from which air for analysis was drawn :—(1) Centre of Equalizing Chamber; (2), (3) and (4) About breathing level in Debating Chamber; (5) 6 inches below ceiling of Debating Chamber.

burners, the products of combustion from which are carried away by the flues shown in Fig. 2 into a main flue. These burners assist the outflow of vitiated air from the Chamber, and further a strong pull is maintained on the openings in the ceiling by means of coke fires at the base of the upcast shafts by which the air eventually escapes to the open. The arrows in the illustration indicate the courses taken by the vitiated air. The bulk of it is discharged up the shaft above the Commons Lobby, but a portion passes through downcast shafts which lead to the base of the Clock Tower, and escapes up the latter.

The chemical analyses of the air in the Chamber comprised merely the estimation of the proportion of carbonic acid present at various points, and incidentally the proportion of moisture was ascertained. With regard to the choice of method for the determinations, on the one hand the Committee wished the results obtained to be not merely sufficiently accurate for most practical purposes, but as accurate as it was possible to obtain by any method of estimation, and on the other hand the taking of samples had to be carried out without violating the historic rule against the admission of strangers to the floor of the House during the sittings. The gravimetric method of Haldane and Pembrey[1] was chosen as fulfilling these conditions. It presented the further advantage of affording facilities for making simultaneous observations on the bacteriological condition of the air without the use of additional or independent measuring and sampling apparatus. The bacteriological determinations were carried out by Dr G. S. Graham-Smith, and he is giving an account of them in another part of this number of the *Journal of Hygiene*[2]. The arrangements adopted for the determinations on the air at the breathing level in the body of the Chamber may be briefly described as follows.

Lead or "composition" gas piping was laid from the equalizing chamber through holes beneath the seats to each of the places at which it was decided to make an examination of the air. The places actually selected are approximately indicated by the figures 2, 3 and 4 in Figs. 2 and 3. The end of the pipe within the Chamber was bent over so that for about six inches it was at an angle of about 15° to the horizontal. This end protruded, in the case of two positions, over a vacant seat on the benches, and at the appointed time for the commencement of a determination, Members, who had offered their aid, attached to the end of each pipe a glass tube packed with sterilized glass wool for filtering out the germs in the air which was drawn through

[1] *Philosophical Magazine*, April, 1890. [2] See p. 498.

the pipe. The other position was against the wall on one side of the chamber about 5 feet from the floor level, and the end of the pipe was bent over, and the germ filter attached in the same manner. These positions were varied slightly in the different sets of determinations. The piping was carried to convenient positions in the equalizing chamber for stands to support the tubes for the absorption of carbonic acid and moisture, as well as the aspirators which served to suck the air at the desired rate of flow from the Debating Chamber through the germ filters, piping, and train of absorption tubes, and to measure the volume of air so drawn through the apparatus. In addition to the three sets of tubes, piping, and aspirators already referred to, a similar set of apparatus was established in the equalizing chamber to draw air from that chamber in order to obtain, for comparative purposes, determinations on the fresh air about to be supplied to the Debating Chamber. This position may be conveniently numbered " 1," as it represents the incoming air to the Chamber of the House. A fifth set of apparatus was established on the roof of the Chamber, the end of the composition piping being passed in to a point about six inches below the ceiling near the bar end of the Chamber. The object of this set of apparatus was to obtain determinations on the air as it was about to leave the Chamber.

The germ filters were prepared, and the subsequent bacteriological investigations carried out, by Dr G. S. Graham-Smith (see p. 499).

A rate of flow of about one litre per minute has been found most convenient for the carbonic acid determinations with the form of absorption tubes employed, and a suitable duration for a determination was known to be from 30 to 40 minutes. The same rate of flow also answered for the bacteriological investigation. The aspirators therefore had to have each a capacity of fully 40 litres, and to be capable of affording measurements accurate on this volume to within 0·5 per cent. In order to avoid measuring large volumes of the effluent water from the aspirators each time they were used, the total capacity of each aspirator was accurately determined in the first instance, and, the aspirators being chosen of such a size that their capacity exceeded by only a little the volume required for each determination, the actual volume of air drawn into them was ascertained by difference, *i.e.* by measuring the volume of water—a few litres only—remaining in the aspirator and deducting it from the previously ascertained total capacity. Suitably chosen carboys, fitted with rubber plugs and connexions of glass tubing, served as the aspirators, and in addition to the total

capacity being determined and marked on each, for convenience of controlling the rate of flow each was provided with a litre scale, which enabled the number of litres of air drawn in to be approximately read off at any moment.

The order in which the component portions of the complete train of apparatus were set up was—reckoning from the place from which the sample of air was drawn—as follows: (1) The bacteria arresting tube, which was unsealed and attached at the moment of commencing a determination. (2) The length of lead or "composition" gas piping leading from the sample intake to the bench on which the absorption tubes and aspirator were set up. (3) The double-limbed absorption tube, charged with pumice saturated with sulphuric acid, for the absorption of moisture. (4) The first or principal double absorption tube, containing in one limb soda-lime, and in the other sulphuric acid pumice, for the absorption of the carbonic acid. (5) The second or guard tube, similar to the last, for the absorption of traces of carbonic acid which might escape absorption in the preceding tube. (6) The aspirator, arranged to give a flow at a rate of about one litre per minute.

Five of these sets of apparatus were used simultaneously on four occasions, *i.e.* twice on each of two evenings. There were therefore required for carrying out the determinations (1) five double absorption tubes for the absorption of moisture, and one tube similarly prepared and charged, against which each of the five tubes was weighed; (2) five principal double absorption tubes for the absorption of the carbonic acid, and five similar guard tubes, as well as a similarly prepared and charged tube against which each of these ten tubes was weighed. A covered box, with numbered places for the reception of the tubes, was used for carrying them to and from the laboratory and the House of Commons. The three absorption tubes in each set were connected up shortly before the commencement of a determination by means of short lengths of sound well-fitting flexible tubing, and communication between them and the inlet pipe and aspirator was cut off for the time being by means of clips. The whole of the five sets being thus in readiness, the clips were opened, and the aspirators started, all virtually simultaneously, at the moment when the germ filters had been attached to the inlets of the pipes at a signal preconcerted with the Members who had charge of the filters in the Chamber. The filters were removed and plugged, and the aspirators stopped simultaneously, after running from 30 to 40 minutes, on another pre-

concerted signal being given. The whole was carried out so that very few Members in the Chamber except those who were co-operating were aware that the tests were in progress. After the aspirators had been stopped, the absorption tubes were disconnected, and returned to their places in the box, which was then taken to the laboratory for the weighings to be made, while the volumes of air aspirated were accurately ascertained in the manner already described.

The tests were carried out on the 7th and 21st July, 1902, when the attendance of members in the Chamber was quite up to the average. From 200 to a little over 300 persons were present on these occasions in the body of the Chamber and galleries. The first set of determinations on each date terminated shortly before 7.30 p.m. (when the House adjourned for dinner), and the second set about 11.15 p.m. Each set covered a period of from 30 to 40 minutes, as already stated. The outside air was clear on both occasions, and the cotton-wool filter was, therefore, not in action.

The following details of one set of determinations will indicate the magnitude of the actual weighings and measurements, and the degree of accuracy attained in working:

Position No.	Volume of air aspirated. (Corrected to normal temp. and pressure) litres	Increase in weight of			Moisture. Grammes per litre of air	Carbonic acid. Volumes per 10,000 volumes of air
		Moisture tube grammes	No. 1 CO_2 tube grammes	No. 2 CO_2 tube grammes		
1	39·32	0·3152	0·0262	+ 0·0005	0·00802	3·39
2	38·55	0·3106	0·0357	+ 0·0001	0·00806	4·71
3	41·35	0·3397	0·0335	+ 0·0002	0·00821	4·12
4	43·40	0·3510	0·0305	+ 0·0002	0·00809	3·58
5	42·28	0·3526	0·0448	+ 0·0005	0·00834	5·39

It will be seen that the alteration in weight of the second or guard tube for absorbing carbonic acid was so small as to be within the possible error in weighing. In other sets the alteration was occasionally in the opposite direction, *i.e.* the tube had apparently lost in weight by 0·0002 gramme. The changes in weight of this second or guard tube, the primary object of which was merely to indicate whether the first tube had absorbed the whole of the carbonic acid in the aspirated air, were therefore disregarded in calculating the results shown in the last column. The connexions had to be made in the equalizing chamber, which directly communicates with the Debating Chamber by gratings,

rapidly, while the House was sitting, without disturbing the Members either by noise or reflection from a strong light. The time between the two sets of determinations on one evening was none too much for finishing the one set and being ready for the next, as the author deemed it expedient in order to avoid possible confusion to make all the weighings himself. There was less than three hours available for disconnecting the absorption tubes, one set of which was on the ceiling of the Chamber apart from the others, conveying them to the laboratory ½ mile away, making the fifteen weighings, returning with them to the House and connecting them up in the proper order.

Results of Determinations.

Volumes of Carbonic Acid in 10,000 volumes of Air.

	7th July, 1902		21st July, 1902	
Position	First Period 6.30 to 7.10 p.m.	Second Period 10.35 to 11.5 p.m.	First Period 6.48 to 7.30 p.m.	Second Period 10.32 to 11.18 p.m.
1. Centre of Equalizing Chamber, representing the air before it passes into the Debating Chamber	3·74	3·21	3·14	3·39
2. Against the wall on Ministerial Side of Debating Chamber	5·22	4·21	4·72	4·71
3. Ministerial Side, over empty seat in middle row of benches, near gangway	5·17	4·79	3·35	4·12
4. Opposition Side, over empty seat in middle row of benches, near gangway	5·23	5·04	4·96	3·58
5. About 6 inches below ceiling of Debating Chamber, near Bar end	5·60	4·82	5·29	5·39

The results obtained, which are shown in the accompanying table, may now be referred to. Position 1, from which air was drawn for analysis, was in the centre of the equalizing chamber. The air here represented the average condition of the air supplied to the Debating Chamber. The mean of the four sets of determinations showed that there was present on the average 3·37 volumes of carbonic acid in 10,000 volumes of air. The maximum figure obtained was 3·74; the minimum 3·14. As pure country air contains on the average 3 volumes, and the air of towns—in the absence of fog—from 3 to 4 volumes of carbonic acid per 10,000 of air, it will be obvious that the air supplied to the Chamber was, in this respect, commendably good.

Positions 2, 3 and 4 were, as already indicated, in the body of the Chamber, one being against the wall on one side, and the other two in corresponding positions among the benches on opposite sides of the House. The positions were altered slightly for the different sets of determinations, and one very low result is explained by the fact that the air was drawn in close to one of the gangways, to which it would pass almost directly from the equalizing chamber. The results obtained at these three positions in the four sets of determinations may be summarized as follows: The mean was 4·59 volumes of carbonic acid per 10,000 volumes of air—an excess of 1·22 volumes over the mean for the incoming air. The maximum results obtained in the 12 determinations showed an excess of 1·83 volumes, and the minimum an excess of 0·19 volume over the result for the incoming air (Position 1) at the same time.

Position 5, near the ceiling of the Chamber, served to indicate the condition of the vitiated air passing away from the Chamber. The mean result for this position was 5·27 volumes, or an excess of 1·90 volumes over the mean result for the air about to enter the Chamber. The maximum result showed an excess of 2·15 volumes, and the minimum an excess of 1·61 volumes, over the result for the incoming air at the same time.

It will thus be seen that the air supply to the Chamber was adequate to avoid a rise of 2 volumes of carbonic acid over that present in the incoming air, except close to the ceiling, where the rise was on one occasion slightly more than 2 volumes. The highest absolute figure obtained was 5·60 volumes close to the ceiling.

In order to indicate how excellent is the renewal of the air of the Chamber which these figures prove, some suggested standards of ventilation and results obtained in other buildings may be quoted. Pettenkofer held that 10 volumes of carbonic acid per 10,000 volumes of air (or an excess of about 6 volumes over the proportion he found in the air of towns) should not be exceeded in the air of rooms, etc. But the observations of Carnelley, Haldane and Anderson on the air in crowded elementary schools showed that a lower limit than 13 volumes per 10,000 could not be fixed for such buildings in 1887, though now improved means of mechanical ventilation might lead to a revision of this limit. Finally the Departmental Committee which reported to the Home Secretary on the Ventilation of Factories and Workshops in August, 1902, concluded that it was reasonable to expect that under ordinary circumstances 10 volumes should not be exceeded in such

places unless gas was burning. This committee recommended that a maximum limit of 12 volumes should be prescribed as a standard of ventilation for factories and workshops when gas was not burning, and that compliance with it should be ultimately enforced on employers.

It will be seen that the ventilation of the Debating Chamber of the House of Commons was shown by the author's determinations of carbonic acid to be exceptionally good, when judged by the standards of ventilation which have received authoritative recognition.

THE MICROORGANISMS IN THE AIR OF THE HOUSE OF COMMONS.

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(From the Pathological Laboratory of the University of Cambridge.)

CONTENTS.

THE investigations described in the present paper were undertaken during the summer of 1902, at the request of the Select Committee on the Ventilation of the House of Commons.

Methods of collecting and cultivating the organisms in the air.

As a result of certain preliminary experiments, it was decided to adopt a slight modification of the method described by Frankland[4] in 1887. The principle of the method is to entangle the micro-organisms in plugs of tightly packed glass wool by drawing known volumes of air through them. An air pump of known capacity, or under certain conditions automatic aspirators, were used for the aspiration of the air through glass collecting tubes fitted with glass wool plugs.

The collecting tubes.

The collecting tubes were made from strong glass tubing ·5 cm. in internal diameter and 12 cms. in length. Each was provided with three plugs of finely divided glass wool (*A, B, C*) kept in position by constrictions in the tubes. The constriction behind *C* was first made and the plug inserted, next that behind *B*, and finally the one behind *A*. The tube was then marked at the end nearest *C* with a blue cross, and was placed inside a stout glass test-tube, whose open end was plugged with cotton-wool, to act as a sterile case when the collecting tube was not actually being used. The blue-marked end of the collecting tube was placed nearest the open end of the test tube, so that when the latter was opened this end should first drop into the hand. In all subsequent proceedings this end was alone handled, and fixed to the tube of the air pump or aspirator, and at the end of the experiment the collecting tube was again placed inside its case in the same position.
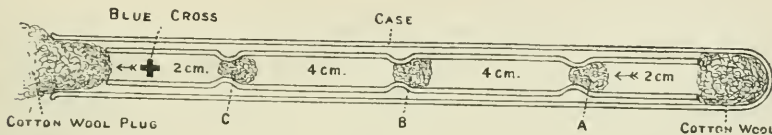
Arrows mark direction of air current.

Before use the collecting tube in its case was sterilised at 160° C. in hot air on these occasions.

During the collection of the sample the air passes through the collecting tube from *A* to *C* and the majority of the organisms are entangled in the plug *A*. On a few occasions a small number passed through to plug *B*, which is used as a control to ascertain what proportions of organisms are carried through *A*.

Plug *C* is to prevent contamination of the other plugs when the collecting tube is being handled.

For the aspiration of the air through these collecting tubes an air-pump was used in the majority of examinations connected to the collecting tube by a rubber pipe about 10 feet in length, so that the disturbance caused by the working of the pump should not influence the air at the place where the samples were being collected.

For experiments in the chamber during a sitting such a method was found to be inconvenient. In this case a known volume of air was aspirated by connecting the collecting tube by means of a length of

lead piping to a graduated carboy from which water was being siphoned into another vessel.

Cultivation of Samples.

After removing the collecting tube from its case, it was cut through half way between *A* and *B*. The free and cut ends were sterilised by passing quickly through a flame, and the plug *A* pushed by means of a sterile wire into a Petri dish containing melted sterile gelatin. The plugs were then broken up and evenly spread out with a sterile platinum needle. The samples were cultivated for 5 days or longer at 20° C. At the end of this time the colonies were counted under a lens, and subcultures made to determine the species of the organisms found.

In this way numerical estimations of the numbers of the organisms per litre were made of the outside air at various places, the Debating Chamber before, and during, debates, the ventilating shaft leading to it, and various Committee, Smoking, and Dining Rooms.

Results of Experiments.

TABLE I.

Experiments on outside air, July 18th.   4·5 litres of air aspirated in each case.

No. of exp.	Place	No. of bacterial colonies	No. of mould colonies	Total colonies	No. of organisms per litre
I	Terrace (ground level)	14	5	19	**4·2*** **(1·1)**†
II	,, (10 ft. from ground)	8	5	13	**2·9 (1·1)**
III	,, (20 ft. from ground)	6	9	15	**3·3 (2·0)**
IV	Clock Tower (half-way up)	6	1	7	**1·5 (0·2)**
V	,, ,, (top)	3	3	6	**1·3 (0·6)**
VI	Peers' Inner Court	16	3	19	**4·2 (0·6)**
VII	Star Court	21	6	27	**6·0 (1·3)**

* indicates number per litre of bacteria and moulds in this and other tables.
† Moulds per litre in brackets ( ) in this and all other tables.

The figures just quoted show that very few bacteria were present in the air at the top of the clock tower (1·3)[1] and that the numbers were about the same half-way up (1·5). At 20 and 10 feet above the ground level there was however a considerable increase, 3·3 and 2·9 respectively, and at the ground level (Terrace) the number was still greater, 4·2.

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[1] All figures, here and elsewhere in the text, record the number of micro-organisms per litre.

Two closed courts were also examined and showed in one case the same number as on the Terrace and in another an increase of 2 per litre (6·0).

At the time when these experiments were conducted there was a slight west wind and the day was bright and moderately sunny. No smoke zone was observed in ascending the clock tower and consequently no experiments were made to ascertain whether greater, or lesser, numbers of micro-organisms are present amongst these floating particles.

Comparison with the observations of other experimenters.

The observations of several investigators[1] have shown that the numbers of the micro-organisms in the outside air are dependent on various factors, such as the time of year, condition of the weather, time of day, and the locality, especially with regard to the height of the latter, and the free circulation of air about it.

Influence of the time of year.

The experiments of Frankland[5], Griffiths[8], and others show that the numbers are least during the winter months and greatest during the summer, reaching a maximum during August. Frankland records the following results from observations on the roof of the Science School, Kensington.

January	·4	August	10·5
March	2·6	September	4·3
May	3·1	October	3·5
June	5·4	November	1·3
July	6·3	December	2·0

Griffiths[8] found in two situations in Lincoln that the maximum was reached in August and that the mean number from November to March was about half that from April to October.

Lincoln.	Top of Hill.	November to March	Mean	·5
		April to October	,,	1·6
	Base of Hill.	November to March	,,	1·5
		April to October	,,	3·4

[1] Although much work has been done by Petri, Miquel and other continental bacteriologists on this subject, the observations here quoted are mostly those of English investigators, as being most suitable for comparison with the present experiments. The method adopted by each is given with the references to the literature at the end. No figures obtained by the simple exposure of plates have been quoted.

Miquel [9] found in the centre of Paris, in Winter 2·8, Spring 8·9, Summer 12·2, Autumn 6·8.

Influence of weather.

Carnelley, Haldane and Anderson [2] found the proportion of bacteria to moulds in the outside air considerably influenced by the condition of the weather:

On still, damp days this ratio was	1
On windy days, ,, ,,	1·3
On still, dry days ,, ,,	2·6
On windy, dry days ,, ,,	14·1.

They conclude that moulds are not so much affected as bacteria by changes in dampness.

Influence of altitude.

Numerous observations have established the fact that the numbers in the outside air rapidly decrease with the height of the place in which the observation is made.

The results of Griffiths' [8] experiments at Lincoln have just been quoted. They show that in winter there are three times as many bacteria at the bottom as at the top of the Hill, and in summer twice as many.

Frankland [6] has made several observations on this point. His figures are :—

Primrose Hill (top) ...		·9
(bottom)		2·4
Norwich Cathedral (top of spire, 300 ft.)		·7
(tower, 180 ft.)		·9
(close) ...		1·8
St Paul's Golden Gallery ...	1·1 ...	1·0 [1]
Stone ,, ...	3·4 ,,	3·7
Churchyard ...	7·0 ...	4·6

Influence of locality.

It has been found, however, that the numbers present in a locality under observation are influenced to a still greater extent by its surrounding, than by any of the factors yet given, the numbers being greatest in crowded districts, and least in more open spaces.

As instances of this, Frankland gives the following figures [7].

Kensington Gardens	1·3	......[6]	2·1 and ·5
Hyde Park	1·8	......	1·8 „ 3·8
Exhibition Road	55·4	......	

On this point Griffiths [8] also made a series of experiments in London.

		July	August
Forest Hill	...	4·2	5·3
City (near Bank)	...	5·6	7·3
Piccadilly	...	5·3	6·4
Near Monument	...	5·8	7·0

In Dundee town, in quiet places, Carnelley, Haldane, and Anderson [2] found a mean of ·8, as against ·1 in the suburbs.

Influence of Day and Night.

The same observers arrived at the conclusion that open spaces give lower results during the night, and that even close places by night give a lower average than open spaces by day, the moulds particularly showing a large reduction.

	Day	Night	
	Open places	Open places	Close places
Dundee [2]	bacteria ·7 } moulds ·5 } = 1·2	bacteria ·15 } moulds ·0 } = ·15	bacteria ·49 } moulds ·04 } = ·53.

They also made certain experiments illustrating the same point in the courts of the Houses of Parliament (p. 504).

Numbers found in the outside air during the present experiments.

The present experiments were made during the month of July, and from a consideration of the observations just quoted the figures might have been expected to be rather high. The weather was fine and sunny (19° C. in shade and 28° C. in sun).

Experiments I—V correspond closely to the results of Frankland [4 and 6] at St Paul's, showing a gradual increase from 1·3 at the top of the Clock Tower to 4·2 on the Terrace level. It has been shown that the numbers in open spaces are not great even in London, and the latter figure may probably be taken as a fair estimate of the numbers near the ground level in the open space about the Houses of Parliament.

Samples taken at 1 p.m. from two closed courts, the Peers' Inner

Court (VI) and the Star Court (VII), give 4·2 and 6·0 respectively. Carnelley, Haldane and Anderson [2] made an examination of the central court of the Houses of Parliament in 1887, finding on

April 19th, 20th	8.30 p.m. ...	9·2
	1.0 a.m. ...	4·4
May 18th, 19th	6 p.m. ...	22·0
	12.30 a.m. ...	4·5

They state that during the earlier experiments the traffic in the streets was very great, and quote these results as showing the great diminution in the numbers of organisms found in the night as compared with the day.   Experiments VI, VII give numbers only slightly higher than those above quoted in the night, but the places in which they were made would not be much influenced by traffic in the streets.

Experiments within the House.

The arrangements for the ventilation of the Debating Chamber have been fully described and figured in the preceding paper by Mr Butterfield.   It will therefore be sufficient to remark here that the air passes upwards from the equalising chamber to the Debating Chamber through a perforated grating covered with string matting and extending over the whole of the free space on the floor, including the gangways.   Part also enters by openings under the seats.   The outlet is through openings in the roof.

As has previously been mentioned, automatic aspirators were made use of in the investigations in the Chamber during sittings.   In each of the four series of observations made at 7 and 7.15 p.m., 10.30 and 10.45 p.m. respectively, five aspirators were simultaneously in use— four situated in the equalising chamber and one on the roof.   One of the former was connected with a collecting tube placed in the equalising chamber under the central grid of the floor of the House, and three by means of long lead tubes passing through the floor of the Debating Chamber with collecting tubes fixed to the backs of the Members' seats. One of these collecting tubes was placed on the third seat on the Government side at the breathing level, and one in a similar situation on the Opposition side.   A third was fixed at a high level behind the Government seats.   A collecting tube connected with a similar aspirator was projected through an opening in the roof over the entrance door.

In taking these samples the collecting tubes were attached to their pipes at a definite time by certain Members, and the aspirators in the

equalising chamber then turned on. After five minutes the aspirators were stopped, the tubes removed, and the volumes of air drawn through the collecting tubes read off.

In the other mechanically ventilated rooms the air is extracted by propeller fans placed in the windows. The inlets are through flues connected with gratings on the walls.

Table II.

Experiments in Ventilating Shaft of chamber, etc., July 18th.
4·5 litres of air aspirated in each case.

No. of exp.	Place	No. of bacterial colonies	No. of mould colonies	Total colonies	No. of organisms per litre
VIII	Air inlet at Terrace	4	1	5	**1·1 (0·2)**
IX	End of first passage	4	0	4	**0·8**
X	In front of scrim cloth	9	0	9	**2·0**
XI	Behind scrim cloth	6	1	7	**1·5 (0·2)**
XII	Inside cotton wool filter (working)	3	1	4	**0·8 (0·2)**
XIII	Equalising chamber	4	0	4	**0·8**
XIV	Debating Chamber over central grid	6	6	12	**2·6 (1·3)**

The results of the examination of the air at various points in the ventilating passages to the Chamber itself are given in the above table and show that but few organisms were present in the entering air, at any rate on the day of the examination. When no debate was in progress very few bacteria were present in the equalising chamber below the central grid (·8), and on the floor level of the chamber itself over the central grid only 2·6 were found under these conditions.

During debates the mean of four experiments in the equalising chamber (XXI, XXII, XXXI and XXXII) shows that there was a considerable increase (8·3) over the number found in the incoming air when no debate was in progress (·8). The position selected below the central grid is, however, the one in the equalising chamber most likely to be contaminated by the dust falling through from the Chamber above. Though the increase is considerable, yet even during debates, the number is low for such a position, and about the same as the average found by Frankland in the outside air of London in summer.

Four sets of experiments were conducted within the Chamber itself during debates, two at 7 p.m. when the House had been sitting 5 hours, and two at 10.30 p.m. when the sitting had lasted 8½ hours (including the dinner interval). The results of these experiments are given in Tables III and IV.

Table III.

Experiments in the Chamber during a debate, July 21st, 7—7.15 p.m.
Temperature 18° C.

No. of exp.	Place	No. of bacterial colonies	No. of mould colonies	Total colonies	Litres of air aspirated	No. of organisms per litre
XV	Government side, third seat	34	14	48	4·5	**10·6 (3·1)**
XVI	,, ,, ,,	18	3	21	4·0	**5·2 (0·7)**
XVII	Government side, back seat	26	5	31	6·0	**5·1 (0·8)**
XVIII	,, ,, ,,	17	7	24	5·5	**4·4 (1·2)**
XIX	*Opposition side, third seat	—	—	—	—	—
XX	,, ,, ,,	18	10	28	4·5	**6·2 (2·2)**
XXI	Equalising chamber, under central grid	25	15	40	5·0	**8·0 (3·0)**
XXII	,, ,, ,,	27	10	37	4·0	**9·2 (2·5)**
XXIII	Roof over entrance door	30	14	44	5·0	**8·8 (2·8)**
XXIV	,, ,, ,,	26	15	41	5·0	**8·2 (3·0)**

* Aspirator failed to work.

XV, XVII, XIX, XXI, XXIII, were carried out simultaneously at 7 p.m. and XVI, XVIII, XX, XXII, XXIV simultaneously at 7.15 p.m. The day was windy and damp. 282 persons were present, namely 176 Members, 66 strangers, 28 reporters, and 12 ladies. A division took place at 6.30, 393 Members being present.

Table IV.

Experiments in the Chamber during a debate, July 21st. 10.30—10.45 p.m.

No. of exp.	Place	No. of bacterial colonies	No. of mould colonies	Total colonies	Litres of air aspirated	No. of organisms per litre
XXV	Government side, third seat	17	11	28	4·0	**7 (2·7)**
XXVI	,, ,, ,,	14	10	24	4·0	**6 (2·4)**
XXVII	Government side, back seat	15	7	22	5·0	**4·4 (1·4)**
XXVIII	,, ,, ,,	11	2	13	2·5	**5·2 (0·8)**
XXIX	Opposition side, third seat	23	6	29	5·5	**5·2 (1·0)**
XXX	,, ,, ,,	13	10	26	4·5	**5·7 (2·2)**
XXXI	Equalising chamber under central grid	26	12	38	4·5	**8·4 (2·6)**
XXXII	,, ,, ,,	22	13	35	4·5	**7·7 (2·8)**
XXXIII	Roof over entrance door	30	5	35	5·0	**7·0 (1·0)**
XXXIV	,, ,, ,,	26	6	32	5·0	**6·4 (1·2)**

XXV, XXVII, XXIX, XXXI, XXXIII were carried out simultaneously at 10.30 p.m. and XXVI, XXVIII, XXX, XXXII, XXXIV at 10.45. 305 persons were present, namely 152 Members, 108 strangers, 26 reporters, and 19 ladies.

They indicate that the organisms are most numerous in the equalising chamber. The numbers near the roof are nearly as great. Within the Chamber itself, the greatest number was found, as would be ex-

pected, on the most crowded side, namely the Government third seat (7·2), next most numerous on the Opposition third seat (5·4), and least at a high level behind the Government benches (4·8).

By taking the highest numbers obtained, namely those in the equalising chamber, as 100% the following ratio is obtained, 91% at the roof, 87% third Government seat, 65% third Opposition seat, and 57% above Government back seat.

Although the actual numbers were excessive, the ratio obtained from the earlier experiments is, with the exception of the third Government seat, almost identical, namely equalising chamber 100%, roof 96%, Government third seat 123%, Opposition third seat 57%, and above Government back seat 53%.

The results of the experiments within the Chamber are given shortly in the annexed table.

TABLE V.

Summary of experiments in Chamber during debates, July 21st.

Position of Tube	Organisms per litre				Mean of all experiments	Ratio
	Early Series		Late Series			
	7 p.m.	7.15 p.m.	10.30 p.m.	10.45 p.m.		
Government side, third seat	10·6 (3·1)	5·2 (0·7)	7·0 (2·7)	6·0 (2·4)	**7·2** (2·2)	87%
Government side, above back seat	5·1 (0·8)	4·4 (1·2)	4·4 (1·4)	5·2 (0·8)	**4·8** (1·0)	57 ,,
Opposition side, third seat	—	6·2 (2·2)	5·2 (1·0)	5·7 (2·2)	**5·4** (1·8)	65 ,,
Equalising chamber	8·0 (3·0)	9·2 (2·5)	8·4 (2·6)	7·7 (2·8)	**8·3** (2·7)	100 ,,
Roof	8·8 (2·8)	8·2 (3·0)	7·0 (1·0)	6·4 (1·2)	**7·6** (2·0)	91 ,,

It is interesting also to observe that the numbers were in most cases remarkably uniform, and that there was no increase at the later time. During the experiments at 6.30, 282 persons were present in the Chamber and Galleries, and at 10.30, 305 persons.

When the experiment in Committee Room No. 9 was made the fans were working, and 150 persons were present. The result showed 20·9 organisms per litre. In Committee Room No. 1 with only 41 persons present, the windows open but no fans working, the mean of two experiments (XXXVII, XXXVIII) gave 34·6.

In these rooms therefore three and five times as many bacteria were discovered respectively as in the Chamber itself.

TABLE VI.

Experiments in Committee, Dining, and Smoking Rooms.
4·5 litres of air aspirated in each case.

No. of exp.	Place	No. of bacterial colonies	No. of mould colonies	Total colonies	No. of organisms per litre
XXXV*	Committee Room No. 9. Fans working, 150 persons present. 1.45 p.m.	42	18	60	**13·3** (4·0)
XXXVI	,, ,, ,,	73	21	94	**20·9** (4·6)
XXXVII	Committee Room No. 1. Fans not working, windows open, 41 persons present. 1.30 p.m.	136	24	160	**35·5** (5·3)
XXXVIII	,, ,, ,,	133	19	152	**33·7** (4·2)
XXXIX	Central Dining Room. 36 persons present. 8 p.m.	153	33	186	**41·3** (8·4)
XL	,, ,, ,,	145	54	199	**44·2** (12·0)
XLI	Members' Smoking Room. 24 persons present. 9 p.m.	90	48	138	**30·6** (10·6)
XLII*	,, ,, ,,	19	20	39	**8·6** (4·4)

* In each of these cases part of the plug was loosened during transit and the result is therefore not reliable.

Samples from the Members' Smoking Rooms showed 30·6 per litre (XLI), being about the same number as found in Committee Room No. 1, but from the Central Dining Room still higher figures were obtained, namely, 41·3 and 44·2, mean 43·2.

In certain samples of dust enormous numbers of micro-organisms were present. These samples were obtained from the Ways and Means Corridor, the Chamber itself, and the corridor outside the Lavatory.

That from the Ways and Means Corridor gave in ·01 grammes 18,944 colonies or about 1,900,000 per gramme.

Comparison of the above figures with the results of other investigations on air within rooms.

It is probably most advantageous to compare the numbers of organisms found in the Debating Chamber, Committee, and other rooms with those found in schools.

It has already been shown (p. 502) that to some extent the numbers, and proportion of bacteria to moulds, depend in such situations on conditions of the weather. There are, however, other factors which influence the numbers in a much greater degree, namely the locality, cleanliness, age, and ventilation of the building, and the amount of disturbance of the atmosphere.

Influence of Locality.

Carnelley[1] has demonstrated that even within buildings the altitude exerts some influence. He found on the average the following numbers in schools situated at various altitudes.

Situation			No. of schools examined			Organisms per litre
High	...	...	25	...	...	95
Medium	...	...	20	...	...	106
Low	...	...	19	...	...	164

Even the slight difference caused by the flat on which the room is situated causes an appreciable difference according to this observer.

Situation			No. of schools examined			Organisms per litre
Downstairs	...	...	28	...	...	122
Upstairs	...	...	28	...	...	62

The same investigator also gives figures to illustrate the influence of open, and closed, situations.

Situation			No. of schools examined			Organisms per litre
Most open	...	...	18	...	...	66
Medium	...	...	18	..	...	84
Least open	...	...	17	...	...	135

(These figures have been inserted to show that some differences have been found to prevail under the conditions mentioned. It is evident, however, that the more highly, and more openly situated, schools are probably the more recent, and cleaner. Upper and lower rooms also may not be occupied to the same extent. In consequence of these considerations too much stress should not be laid on these results.)

Influence of the Age of the Building.

It has been shown by Carnelley[1] and by Carnelley, Haldane, and Anderson[2] that the age of the building has a most striking influence on the numbers found in the school rooms.

		Years opened		No. of cases		Organisms
(1)	Country Schools ...	more than 22 years	...	24	...	81
		between 17—13 ,,	...	16	...	65
		within 1 year	...	3	...	36
	Schools in suburbs and county towns	more than 22 years	...	4	...	199
		between 15—9 ,,	..	41	...	97
		within 1 year	...	6	...	65
(2)	Schools in Dundee	Built before 1866	.. ...	7	...	311
		,, between 1875—1880 ...	...	20	...	150
		,, ,, 1880—1885 ...	...	5	.	38

(Experiments made in 1886.)

Influence of Cleanliness.

The two sets of observers from whom the last figures were quoted have also demonstrated that the cleanliness, or otherwise, of the rooms of schools and dwelling houses is a factor of some importance in determining the numbers present.

Schools			Natural ventilation			Mechanical ventilation
Cleaner	...	...	91	...	...	3
Average	...	...	125	...	...	16
Dirtier	..	..	198	...	...	30

Influence of Ventilation.

Of all the various factors which have a bearing on the number of micro-organisms present in the air of rooms, probably ventilation is the most important. It has been found, however, that mechanical ventilation at the time of the experiment is of little importance, but the following tables from Carnelley [1] show that the effect of habitual mechanical ventilation is well marked.

			No. of schools examined	Organisms
Town schools (Aberdeen).	Natural ventilation	...	17	136
,, ,, (Dundee).	,, ,,	...	19	152
,, ,, (Aberdeen).	Mechanical ,,	...	3	20
,, ,, (Dundee).	,, ,,	...	4	17

The immense difference made by efficient mechanical ventilation is also well brought out by the observations of Carnelley, Haldane and Anderson [2]. They found in schools with

Natural ventilation	...	28 cases	...	152·1 organisms
Mechanical ,,	...	18 .,	...	16·58 ,,

Sources of the micro-organisms found in the Air.

The latter observers [2] have made it clear that bacteria are not given off in the ordinary respiration of healthy persons, and that the number of micro-organisms given off by the skin and clothes of persons actually present in a room is small compared with those coming from the dust in the room. They have also shown that in dwelling rooms the micro-organisms decrease as the cubic space increases. They quote the results of Hesse who has proved that when a room is left quiet the micro-organisms settle out in a few hours so that the air becomes comparatively free.

Certain observations made by them demonstrate the fact that the disturbance of dust as by stamping of feet causes an enormous increase of organisms, in one case from 11 to 150. The making of the beds in an Infirmary resulted in an increase from 2·8 to 28.

They are of opinion, however, that the ordinary movements even of many persons in a room are not sufficient to produce any marked change in the numbers of organisms.

Dust from the House was shown to contain about 2,000,000 organisms per cubic centimetre. It is obvious from such figures how great a contamination of the air might be brought about by the disturbance of this dust in sweeping.

The Debating Chamber.

In view of the data concerning the numbers of the micro-organisms in the air of schools, the open situation, cleanliness, and mechanical ventilation of the Debating Chamber, together with the fact that comparatively little disturbance of the atmosphere is caused, would lead to the expectation that few bacteria would be found in the air of the Chamber in spite of the age of the building. Experiments XV—XXXIV show that the mean number in the Chamber itself in the four sets of experiments was only 5·8, a number only slightly in excess of that of the outside air, and even slightly lower than the average found by Frankland in July for outside air. Comparison with the figures already given brings out the fact that in the chamber there are fewer organisms than in any of the mechanically ventilated schools with one exception. Detailed examination of the figures quoted by Carnelley [1] shows that of the twelve mechanically ventilated school rooms examined by him three had lower figures, and of the 131 naturally ventilated rooms only two had a smaller number.

It may, therefore, be said that the air of the Chamber is, as regards the micro-organisms present, exceptionally pure.

Committee, Dining, and Smoking Rooms.

With one exception, namely the method of ventilation, these rooms are under the same conditions as the Chamber in regard to the factors which have an influence on the numbers of the micro-organisms, and it might be fairly deduced that if the ventilation were as efficient, the organisms would be as few.

During the taking of the sample in Committee Room No. 9 (XXXVI) 150 persons were present, and the fans were working. The

number found, 20·9, though considerably greater than the mean in the Chamber, is very little above that given for mechanically ventilated schools.

In Committee Room No. 1 (XXXVII, XXXVIII) only 41 persons were present, the windows were open, but no fans were working. The mean of two experiments showed 34·6 bacteria. The mean of experiments in the Central Dining Room showed 43·2 (XXXIX, XL), and the Members' Smoking Room 30·6 (XLI).

In all these rooms with the exception of Committee Room No. 9, the numbers considerably exceed those quoted for mechanically ventilated schools, and even No. 9 is slightly in excess of the standard of purity suggested by Carnelley, Haldane and Anderson [2] for dwelling houses and schools. These observers consider that the limit of 20 organisms per litre should not be exceeded, nor the ratio 30 of bacteria to moulds. The latter ratio in these observations is, however, not exceeded, it being only 3·6.

From the foregoing considerations it would seem that the unsatisfactory condition of these rooms in regard to the number of bacteria is due to insufficient ventilation.

In the passages through which the air passes to the Chamber very few organisms were found, as shown in experiments VIII—XIII, and near the floor of the Chamber when no sitting was in progress (XIV) only 2·6, about half the mean number during sittings, were discovered.

Identification of Bacteria.

After the numerical calculations had been made from the gelatin plates, subcultures were sown into gelatin tubes from dissimilar colonies. These subcultures were grown at room temperature, and subsequently each was planted in the following media, gelatin stabs, potato, broth, glucose broth, nitrate and rosolic acid solutions, and milk. In these several media the morphological appearances of the organisms, the characteristics of their growth, and their power of producing acid, reducing nitrates, decolourising rosolic acid and curdling milk were noted. Further, each organism was tested for its power of forming gas, and finally the result of subcutaneous inoculation of 1 c.c. of a broth culture, grown for 48 hours or more, into a guinea-pig was observed.

Species which occurred on agar plates grown at 37° C. were similarly tested. (See Parliamentary paper referred to by Butterfield in the preceding paper p. 486 for details of species found.)

No bacteria were found which showed any discernible growth on blood serum in 24 hours at 37° C. and only two which grew anaerobically on agar at the same temperature. The results in these cases were probably due to the small quantities used for these cultivations, and in the latter also to the fact that in the majority of instances the tubes were soon overgrown with moulds.

The majority of the colonies which appeared on gelatin consisted of micrococci of various kinds. Bacilli were not so common. No spirilla were found, and moulds and yeasts were not identified.

From a considerable number of pure cultures, 20 species of bacilli and 27 of cocci were isolated. These, however, probably do not include all the species which were present on the plates, as some must have been overlooked in making the subcultures.

By the methods of cultivation employed in the course of these experiments no pathogenic bacteria associated with specific diseases in man were discovered, but certain organisms were found capable of producing various lesions and even death in guinea-pigs when injected subcutaneously[1].

Conclusions.

1. The number of micro-organisms in the open space surrounding the Houses of Parliament is comparatively small (4·2 per litre on the ground level).

2. At the top of the Tower there are only about one-third of the number found at the ground level.

3. From a bacteriological point of view the air in the Debating Chamber during a sitting is under the circumstances remarkably pure (5·8 per litre as a mean of 11 experiments).

4. The numbers found in the Committee, Dining, and Smoking Rooms were several times as great as in the Chamber (32·3 per litre as a mean of 6 experiments).

5. No organisms associated with specific diseases in man were isolated, and only a few pathogenic to animals.

[1] See p. 512.

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NOTE ON THE OCCURRENCE OF MALARIAL FEVER IN PLACES USUALLY FREE FROM ANOPHELES.

By JOHN CROPPER, M.A., M.D.

THE above heading sufficiently indicates a difficulty which has doubtless often occurred to those who have studied the subject of the agency of *Anopheles* in the causation of malaria. In some cases this difficulty has seemed so formidable that disbelief in any such agency has been boldly professed. The following observations, most of which I owe to the kindness of Dr Gould, who succeeded me at Acre in Palestine, will help to explain the occurrence of malaria at places where there would seem to be little or no breeding-ground for *Anopheles*, and prolonged observation by residents has not revealed the presence of the perfect insect.

I. As mentioned in a former paper in this *Journal* (Vol. II. p. 47, 1902) on the Geographical Distribution of Malaria in Upper Palestine, though resident for some years in the town of Acre, I had never been able to find a single specimen of *Anopheles*. This year (1903), Dr Gould had two patients—boys of his native dispenser—ill with malaria. The parasite at various stages was clearly demonstrated in their blood, and the facts allowed of only one interpretation, viz. that in at least one case the disease had been caught in the town. *Anopheles* mosquitoes were found in the room in which they lived. It is uncertain where these were bred, but last winter was exceptionally wet, and the water covered the plain within less than half-a-mile from the town, near the wall of which the house is situate. As I mentioned in the paper just referred to malaria occurs in Acre itself, but comparatively rarely, though common enough a mile away.

II. At Shefa Amr, 3 hours from Acre, situated on low lying chalk hills, well raised above the plain and within reach of no running water, except after heavy rain, two English ladies had malaria, one after the other. Though imagines of *Anopheles* were not found, yet in the water

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from an open cistern, brought for washing, larvae of *Anopheles* were found quite recently by Dr Gould.

I had the privilege of Dr Gould's help when in the country two years ago, and know how well acquainted he is with the larvae of *Anopheles*.

III. The third place to be mentioned is Nablous, where I spent some months this summer. In 1901 Dr Gould found *Anopheles* larvae in a shallow cistern used to catch the rain water before it is allowed to run into the main cistern of the parsonage belonging to the Church Missionary Society. This year an examination of several of the open cisterns in houses in the town to which I went to treat patients, gave a negative result, nor was I more successful outside the town. On examination of the above-named cistern however I succeeded in getting 9 *Anopheles* without special apparatus, the vast majority of the species being *Culex* or *Stegomyia fasciata*.

Cases of malaria are quite well known in the town but are very infrequent considering the large number of outpatients treated at the Hospital. Splenic enlargement is also rare. Amongst the English ladies working in the Mission a few attacks of malaria have occurred, their house being but a few yards away. More recently two *Anopheles* larvae were found at the new Hospital, with many *Culex*.

Within 4 hours of Nablous to the east is the Ghor or Jordan Valley, notorious for its unhealthiness; and within 9 hours is Beisân, than which few places are more deadly. Patients and visitors to the town often come in from these parts, and thus the malarial parasite, which would otherwise die out, is furnished. So it is probably with many, if not all places in the Tropics not essentially malarious, *i.e.* not furnished with an abundant supply of *Anopheles*.

IN MEMORIAM.

EDMOND NOCARD.

THE untimely death of Professor Nocard comes as a severe blow to medical science throughout the world. A glance through the long list of important papers which he published, more especially of recent years, sufficiently attests his enormous industry and exceptional talent as an experimental investigator. His work followed the traditional lines of the school of Pasteur in that it began with an attack upon problems of purely scientific interest in the domain of biology, and resulted in numerous practical applications of far-reaching utilitarian and economic importance.

Edmond-Isidore-Étienne Nocard was born 29 January, 1850, at Provins (Seine-et-Marne). His father was a wood merchant. Nocard pursued his early studies at Provins, and, in 1868 to 1873, studied at the Veterinary School of Alfort, his course of study being prolonged through the interruption of the Franco-German war, in which he served as a volunteer in the 5th Lancers. For five years following his graduation, he held a clinical appointment in the Veterinary School of Alfort, becoming then Professor of Pathology and Clinical Surgery. He married Mademoiselle Josias in 1875 and by her, who died in childbirth, had one daughter who remained his companion to his death.

Nocard began publishing in 1876, but first came into prominence through his association with the Pasteur School, which he joined through the influence of his friend Professor Roux. He was especially welcome there because of his knowledge of veterinary science. In 1883, he, together with Straus, Roux and Thuillier, went to study cholera in Egypt, where one of the devoted workers—Thuillier—died of the disease. In 1887 he became Director of the Alfort School and in the same year exchanged his chair for that of contagious diseases, sanitary police and jurisprudence, a position for which he was eminently fitted by virtue of his bent and talents. In 1891 he resigned the Directorship so as to more

fully devote himself to the duties of his chair and also to research. Upon the organization of the Institut Pasteur in Paris, he was appointed a chief of service in that Institution, and soon became an active and indispensable member of various societies and of national and international committees or councils dealing with matters of public health, infectious diseases, agriculture and veterinary science. He soon gained official recognition at home, being decorated with the Cross of the Legion of Honour. In 1886 he was chosen a member of the Académie de Médecine. His reputation extended, however, throughout the scientific world, as his important investigations became generally known. He travelled extensively, and was called in as adviser by foreign governments, two of which, those of Belgium and Italy, rewarded him with decorations for his services.

His most important contributions to science were on bovine peripneumonia, published in collaboration with Roux; on the relation of avian to mammalian tuberculosis; on the use of tuberculin in the diagnosis of tuberculosis in cattle; on the use of mallein in the diagnosis of glanders in horses, together with observations regarding the curability of the disease, its diagnosis, and prophylaxis. His investigations on tuberculin and on mallein were of great use in establishing the value of these means of diagnosis in practice. His studies on the preventive use of anti-tetanic serum in horses were likewise of great practical and economic importance, and should have a bearing upon the prevention of tetanus in man. Important also were his investigations upon contagious mammitis of cows, and of gangrenous mammitis in milch-ewes, upon bovine farcy, rabies, piroplasmosis, trypanosomiasis, ulcerative lymphangitis in horses, black-quarter, foot-and-mouth disease, sheep-pox, in short on practically every subject to which he directed his great abilities. A great deal of his work has found a place in a book entitled "Les maladies microbiennes des animaux," published together with Leclainche, and which has appeared this year in its third edition. Nocard's papers appeared chiefly in veterinary journals ("Recueil de méd. vétér."; "Bulletin de la soc. centr. de méd. vétér.), and in the "Annales de l'Institut Pasteur." He was also a collaborator of the "Archiv vétérinaire" and of the "Dictionnaire de méd. vétérinaire." The accompanying bibliography includes his more important papers which have appeared since the year 1886.

In the debates at International Congresses, which he frequently attended, his ready wit and charm of exposition impressed his hearers. What he said was always listened to with attention, for he never spoke

unless he had light to throw upon the subject, the light of a finely critical mind replete with knowledge.

Nocard died, 2 August, 1903, at Saint-Maurice, having almost attained the age of 53 years. His death was preceded by a painful illness of three weeks. His burial was attended by a large number of prominent men of science, who, in accordance with French custom, held eloquent orations over his grave, the words uttered bearing ample testimony to the high esteem and affection in which he was held by all who knew him. His friend of twenty years, Professor Roux, speaking for the members of the Institut Pasteur, said :—

"Mieux que personne j'ai pu apprécier sa puissance de travail, son esprit d'invention, et l'admirable clarté de son intelligence. Ces qualités sont évidentes dans chacun de ses travaux, tant dans la conduite des expériences que dans la façon dont elles sont exposées. Elles se manifestaient dans sa manière de parler et jusque dans l'aspect de sa personne. La limpidité de son regard, le sourire de sa bouche avertissaient, dès l'abord, de son honnêteté et de sa bonté....Nocard a été terrassé d'un coup, à la veille de produire des travaux plus importants encore que ceux qu'il avait déjà faits. Sa mort, qui est un malheur pour notre pays, sera déplorée bien au-delà de nos frontières. Mais elle cause une véritable stupeur dans la maison de Pasteur. Car pour nous, Nocard n'était pas seulement le collaborateur précieux, il était l'ami sûr, le conseiller des heures difficiles, le compagnon fidèle et charmant."

The Editors of the *Journal of Hygiene* are indebted to Professor Metschnikoff for the excellent likeness which serves as a frontispiece to this number, the photograph being one of recent date.

G. H. F. N.

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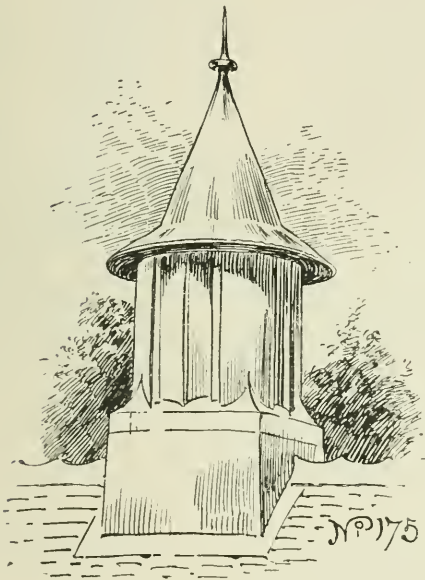
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